A Review on Biomarkers: As a Potential Diagnostic Tool

Keywords: Antecedent biomarker, Diagnostic biomarker, Cancer biomarker, Cardiac biomarker, Variability, Validity, Reliability

ABSTRACT

A biomarker is a biochemical feature which is used to measure the progress of a disease or effects of treatment. They can be introduced into an organism to examine organ function or any other aspects of health. Biomarkers provide a dynamic and powerful approach for understanding observational and analytical epidemiology, randomized clinical trial, screening, diagnosis, and prognosis. Biomarkers can reflect the entire spectrum of diseases from the earliest manifestation to the terminal state. This review describes major uses of biomarkers in clinical investigations. Careful assessment of the validity of biomarkers is required concerning the stage of the disease. Various issues that affect the analysis of biomarkers are discussed.
INTRODUCTION

Biological markers (biomarkers) have been defined by Hulka and colleagues\(^1\) as “cellular, biochemical or molecular alterations which can be measured in biological media such as human tissues, cells, or fluids.” The definition has been expanded as naturally occurring molecules, genes, hormones, by which biological characteristics can be measured and evaluated as the indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention\(^2\). In practice, biomarkers include the use of tools and technologies that can aid in understanding prediction, cause, diagnosis, progression, regression, or outcomes of the treatment of diseases. Biomarkers indicate the normal as well as the abnormal process taking place in the body and may be a sign of underlying disease. For example, in the case of the nervous system, there is a wide range of techniques that are used to gain information in both healthy as well as diseased state. These may include the measurements directly on biological media (e.g., blood or cerebrospinal fluid) or measurements such as brain imaging which do not involve sampling of biological media directly but measures the changes in the composition or function of the nervous system\(^1,3\).

Different type of biomarkers has been used by generations of epidemiologists, physicians, and scientists to study various human disease. The application of biomarkers in the field of diagnosis and management of cardiovascular disease, infections, immunological and genetic disorders, and cancer are well known\(^1,3\). Their use in research has a direct measurement of exposures in the causal pathway of disease that have the potential of providing information on the absorption and metabolism of the exposures\(^4\). The rapid growth in molecular biology and laboratory technology has expanded where the application of technically advanced biomarkers will soon become even more feasible\(^5,8\). Molecular biomarkers in the hands of clinical investigators provide a dynamic and powerful approach for understanding the spectrum of neurological diseases with obvious applications in analytic epidemiology, clinical trials and disease prevention, diagnosis, and disease management \(^6\). In drug development and clinical trial, biomarkers can be used to measure the effects of an investigational drug on people during the clinical trials, to identify the population for study, monitoring therapeutic response, and to identify side effects\(^5-7\).
Figure No. 1: Role of biomarkers

TYPES OF BIOMARKERS

A biomarker has been classified by Perera and Weinstein based on the sequence of events from exposure to disease (FIG 1) \(^3\). Biomarkers readily tend themselves to epidemiological investigations and are useful in the investigation of the natural history and prognosis of a disease.

Figure No. 2: Disease pathway and the impact of biomarker
Table No. 1: Capabilities of Biomarkers

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Capabilities of Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Delineation of the events between exposure and disease</td>
</tr>
<tr>
<td>2</td>
<td>Establishment of dose-response</td>
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<tr>
<td>3</td>
<td>Identification of early events in the natural history</td>
</tr>
<tr>
<td>4</td>
<td>Identification of mechanisms by which exposure and disease is related</td>
</tr>
<tr>
<td>5</td>
<td>Reduction in misclassification of exposures or risk factors and disease</td>
</tr>
<tr>
<td>6</td>
<td>Establishment of variability and effect modification</td>
</tr>
<tr>
<td>7</td>
<td>Enhanced individual and group risk assessments</td>
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The events occurring between exposure and disease, biomarkers have the potential to identify the earliest events in the natural history, reducing the degree of misclassification of both disease and exposure, and opening a window to potential mechanisms related to disease pathogenesis, accounting for some of the variability and effect modification of risk prediction. Biomarkers provide insight into disease progression, prognosis, and response to therapy. There are two major types of biomarkers:

- Biomarkers of exposure - Which are used in the prediction of risk.
- Biomarkers of disease - Which are used in screening and diagnosis and monitoring the progression of the disease.

Biomarkers used in risk prediction, screening, and diagnostic tests offer distinct and obvious advantages. The classification of many diseases is based either on standardized clinical criteria or histological diagnosis. Biomarkers have the potential to identify diseases from an early stage, to provide a method for homogeneous classification of disease, and to extend the knowledge base concerning the underlying disease pathogenesis. These advantages have a direct application to all the types of clinical investigation, from clinical trials to observational studies in epidemiology. In epidemiological (or quasi-experimental) investigations, biomarkers improve validity while reducing bias in the measurement of exposures (or risk factors). The use of biomarkers improves the sensitivity and specificity for the measurement of the exposures or risk factors.
Molecular biomarkers have the potential to identify individuals who are susceptible to disease. Molecular genetics already had an impact on neurological practice, which leads to improved diagnosis. Classification of populations based on the degree of susceptibility based on such biomarkers produces greater accuracy than relying on historical definitions of susceptibility. For example, a biomarker will allow the stratification of a population-based on a specific “genotype” which is associated with a disease rather than relying on a report of the “family history” of the disease. The ability to quantify “susceptibility” in this way is an extremely important method for estimating disease risk among various populations.

**BIOMARKERS OF EXPOSURE OR ANTECEDENT BIOMARKERS**

**Environmental exposures, effect modifiers, or risk factors**

When a disease is suspected and is resulting from toxic exposure, researchers would naturally measure the degree of exposure. External exposure is the measured concentration of the toxin in an individual’s immediate state of the environment. While questionnaires offer a historical account of the exposure, direct measurement of the alleged toxin present in the air, water, soil, or food which can provide accurate information regarding the “dose” of the exposure. Measurement of the external dose provides the basis to understand the relationship to the disease processes, and the measurement of “internal” dose may provide more accuracy.

When a toxin is identified in tissues or body fluids it becomes a biomarker for the internal dose. A biomarker that measures a “biologically effective dose” generally indicates the amount of toxin or chemical, measured in the target organ or its surrogate. Lead exposure is an example. The pharmacokinetic properties of the toxin or chemical become important to consider in the measurement of internal dose because several body fluids could be used based on the pharmacological properties of the agent. Some chemicals such as halogenated hydrocarbons are stored in adipose tissue while others, such as organophosphate pesticides, are measured in blood or urine.

Most biomarkers of exposure measure antecedent factors that are thought to modify (increase or decrease) the risk of developing the disease being investigated. The greatest advantage of a biomarker of exposure over the history of exposure is that it estimates the actual “internal” dose of the exposure. This would improve the precision in the measurement of any risk factor by adding both internal and external validity. Biomarkers are useful in the cross-sectional investigation of acute disease due to the pharmacologic properties of the chemical or toxin.
Genetic susceptibility

The epidemiologic analysis examines familial aggregation and assesses genetic and environmental contributions to disease by using life table methods and recurrence risk. Mutations in genes that can result in Mendelian forms of the disease are typically deterministic. Most adult-onset degenerative diseases of the nervous system are related characteristics, heritable, and environmental are likely to be a composite. The correlated combinations of these features constitute the trait or disease. Therefore, these types of antecedent biomarkers may be directly involved in the etiology or not. In some cases, the genetic variant is neither necessary nor sufficient to cause the disease. However, they can be powerful antecedents at any stage of the disease pathway, these antecedent biomarkers exist before the disease or will be independent of other exposures. They improve the precision in the measurement of other associated factors because they may be synergistic or antagonistic. Variations in several genes can show susceptibility to Parkinson’s disease, which is also related to environmental risk factors. Once if established, a specific genotype might be used to predict an association with a particular environmental toxin.¹²

Intermediate biomarkers

Some biomarkers may represent direct steps in the causal pathway of a disease and are therefore strongly related to the disease. Others are related in an indirect way to the cause. A biomarker could be depending on a known or unknown factor to cause disease. The biomarker could also be related to an exposure that had been identified already or which represents an alteration caused by the exposure that can result in disease. The most precarious situation is when the biomarker is related to some unknown factors that are also related to the exposure. This type of confounder, if it is unidentified, can decrease the validity of the association between the biomarker and the disease.

BIOMARKERS OF DISEASE

Screening, diagnostic tests, and prognosis

Biomarkers depicting prodromal signs enable early diagnosis or allows for the outcome of interest to be determined at a more primitive stage of the disease. Blood, urine, and cerebrospinal fluid provide the necessary biological information for the diagnosis. In these conditions, biomarkers are used as an indicator of a biological factor that represents either subclinical manifestation, stage of the disorder, or a surrogate manifestation about the
disease. Biomarkers used for screening or diagnosis also often represent a surrogate manifestation of the disease. The potential uses of this class of biomarkers include the following:

1) Identification of individuals who become affected or who are in the “preclinical” stages of the illness,

2) The decrease in disease heterogeneity in clinical trials or epidemiologic studies,

3) Reflection of the natural history of disease encompassing the various phases of induction, latency, and detection, and

4) The target for a clinical trial.

The improvement of invalidity and precision overcome the difficulty in obtaining such tissues from patients. Most ethical review boards and the healthcare system require adequate follow-ups for individuals that screen to be positive regardless of whether or not they have the disease. Also, treatment should be made available for those who screen positive and should be accessible and acceptable.

Those who screen to be positive and are diseased should be allowed to access treatments and those treatments should be adequate and available. It is useful to remember that the main benefit of screening is primary (before the onset of symptoms) or secondary (early or prodromal detection) prevention of disease. Considers the benefits of conducting a therapeutic trial in patients before an overt manifestation occurs.

Diagnostic tests for neurological diseases are used with increased frequency in clinical research and practice. In the case of diagnosis, the collection of information from various sources, which includes the results from diagnostic tests, helps to achieve the ultimate goal of increasing the probability. Clinical tests are performed, probably less often, for other reasons includes the following:

- To measure the severity of the disease,

- To predict the occurrence of disease,

- To monitor the responses to a particular treatment.
More importantly, biomarkers for disease tend themselves to clinical trials. Another advantage is the reduction in disease heterogeneity in clinical trials or observational epidemiologic studies, which leads to a better understanding of the natural history of disease encompassing the phases of induction, latency, and detection.

**Cardiac Biomarkers**

Cardiac biomarkers are substances that are released into the blood when the heart is damaged. Measurement of these biomarkers is useful in diagnosing, evaluate, and monitor patients with the suspected acute coronary syndrome (ASC)\(^1\). The symptoms of ASC are related to heart attacks and angina, but they may also see with non-heart related conditions. An increase in one or more cardiac biomarkers can identify patients having ACS, thereby allowing rapid diagnosis and appropriate treatment for their condition.

![Figure No. 3: Introduction of biomarkers](image)

![Figure No. 4: Diagnostic and Prognostic biomarker](image)
The cardiac biomarker test is used to help in detecting the presence of ACS and to evaluate its severity so that appropriate therapy can be done. It is important to distinguish a heart attack from angina, heart failure, or other conditions because the treatment and monitoring requirements are entirely different. For heart attacks, prompt medical interventions are crucial to minimizing heart damage and future complications\textsuperscript{18}. Cardiac biomarker tests must be available to the doctor 24 hours a day, 7 days a week with a rapid turnaround time\textsuperscript{19}. Some of the time tests may be performed in Emergency care or at the patient’s bedside. Serial testing of one or more cardiac biomarkers is done to ensure that a rise in blood levels is not missed and also to estimate the severity of heart attack.\textsuperscript{20-21}

The current biomarker test for detecting heart damage of choice is troponin. Other cardiac biomarkers are less specific for the heart. Many other potential cardiac biomarkers are being researched, but their clinical utility has still to be established.\textsuperscript{22-23}

**Table No. 2: Commonly Used Cardiac Biomarker Tests**

<table>
<thead>
<tr>
<th>Marker</th>
<th>What it is</th>
<th>Tissue source</th>
<th>Reason for increase</th>
<th>Time to increase</th>
<th>Time back to normal</th>
<th>When/how used</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>Enzyme-3 different isoenzyme exist</td>
<td>Heart, brain, and skeletal muscle</td>
<td>Injury to muscle and/or heart cells</td>
<td>4 to 6 hours after injury, peaks in 18 to 24 hours</td>
<td>48 to 72 hours, unless due to continuing injury</td>
<td>Performed in combination with CK-MB</td>
</tr>
<tr>
<td>CK-MB</td>
<td>Heart-related isoenzymes of CK</td>
<td>Heart primarily, but also in skeletal muscle</td>
<td>Injury to heart and/or muscle cells</td>
<td>4 to 6 hours after heart attack, peaks in 12 to 20 hours</td>
<td>24 to 48 hours, unless new or continuing damage</td>
<td>Less specific than troponin, used when troponin is not available</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>Oxygen-storing protein</td>
<td>Heart and other muscle cells</td>
<td>Injury to muscle and/or heart cells</td>
<td>2 to 3 hours after injury, peaks in 8 to 12 hours</td>
<td>Within one day after injury</td>
<td>Performed with troponin to provide early diagnosis</td>
</tr>
<tr>
<td>Cardiac troponin</td>
<td>Regulatory protein complex.</td>
<td>Heart</td>
<td>Injury to heart</td>
<td>4 to 8 hours</td>
<td>Remains elevated for 7 to 14 days</td>
<td>Diagnose heart attack, access degree of damage</td>
</tr>
</tbody>
</table>

**Table No. 3: Biomarker Test for Prognosis**

<table>
<thead>
<tr>
<th>BIOMARKER</th>
<th>WHAT IT IS</th>
<th>REASON FOR INCREASE</th>
<th>WHEN/HOW USED</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP</td>
<td>Protein</td>
<td>Inflammation</td>
<td>May help determine risk of future cardiac events in patients who have had a heart attack</td>
</tr>
<tr>
<td>BNP and NT-proBNP</td>
<td>Hormone</td>
<td>Heart failure</td>
<td>Help diagnose and evaluate heart failure, prognosis and to monitor therapy</td>
</tr>
</tbody>
</table>
Cancer Biomarker

Cancer biomarkers are done across the entire health care spectrum, from cancer biological research laboratory to the patient monitoring in the clinic. The applications of cancer biomarkers include the identification of novel therapeutic targets in cancer drug discovery and also uses them as surrogate markers for drug efficacy in a clinical trial. This report describes the factors providing the driving force behind cancer biomarker growth and characterization. Emerging cancer biomarker types and the increasing interest in circulating tumor cells, data on potential DNA, RNA, protein biomarkers under study includes Oncogenes, Germline inheritance, Mutations in drug targets, Epigenetic changes.

Figure No. 5: Cancer biomarker

BIOMARKERS IN DRUG DEVELOPMENT

Increase in cost and complexity of drug development, biomarkers play a key role in the early phases of drug development. Biomarkers can be divided into target, mechanistic, or outcome with varying degrees of linkage to disease or treatment effect. They can be used to determine proof of concept by characterizing the efficacy or safety profiles or determining differentiation from competitor drugs. Clinical validation of biomarker influence in clinical utility directly.
VARIABILITY

Biomarkers have numerous advantages; variability is a major concern. Variability applies regardless of whether the biomarker represents an exposure or effect modifier, a surrogate of the disease, or an indication of susceptibility. Inter-individual variability can result from the amount of an external exposure or a putative toxin is metabolized. For example, individuals exposed to the same chemical may differ in their ability (or inability) to metabolize the agent, or they may have experienced different types of exposures (in the field as compared within the office). Intraindividual variability is usually related to laboratory errors or other conditions, or exposures being unique to the individual spot. Group variability is encountered, but often the desired outcome of a study, it is best when group differences are large. The ability of a biomarker to distinguish between the groups is measured by sensitivity and specificity or similar variance estimates. Consideration of the sources of variability in the measurement of a biomarker decreases the potential for the misclassification of the exposure.

Measurement error is always a concern with biomarkers, other important factors may explain individual or group variability. Interaction with other exposures, drugs, or effect modifiers can increase or decrease the effectiveness of the biomarker under the consideration as exposure or the measure of susceptibility. Variability can be attributed to the effects of
factors such as individual diet or other personal characteristics. The amount of dietary fat influences the biological measurement of lipid-soluble vitamins as well as toxic chemicals. These individual factors should be considered by the investigator to establish the major causes of variability in these investigations.

VALIDITY

Reliability, validity, sensitivity, specificity, ascertainment bias, and interpretation of data using biomarkers should be reviewed. These problems remain when the biomarker is being used as a variable in a clinical trial or an epidemiologic study. Reliability or repeatability is crucial. Laboratory errors can lead to misclassification of exposures or disease if the biomarker is not reliable. Pilot studies should be performed to establish a reasonable degree of reliability. Changes in laboratory personnel, laboratory methods, storage, and transport procedures may affect the reliability of the biomarkers used in any investigation. The evaluation of the validity of a biomarker is complex.

Schulte and Perera12 suggest three aspects of measurement of validity:

1. Content validity, which shows the degree to which a biomarker reflects the biological phenomenon being studied,

2. Construct validity, which pertains to other relevant characteristics of the disease or trait,

3. Criterion validity, which shows the extent to which the biomarker correlates with specific disease and is usually measured by sensitivity, specificity, and predictive power 4.

The use of receiver–operator characteristic curves can provide the tools which are necessary to determine the best choice in terms of sensitivity and false-positive rates, particularly when other tests are used14,15. Most would agree that screening tests would be very desirable for chronic progressive disorders. The purpose of screening is early detection with the hope of preventing the illness altogether. As with other diagnostic methods, sensitivity and specificity tell us the accuracy of the test but not the probability of disease. For that, we need to estimate the predictive values (positive and negative). Positive predictive value (PPV) is the percentage of people with a positive test who are having the disease. This provides us with information about the disease being present if the test is positive. Negative predictive value (NPV) is the percentage of people with a negative test who are not having the disease. Screening, by definition, includes the larger number of individuals without the disease,
generally ascertained via a defined population. Diagnostic tests are designed to improve the clinical diagnoses by enhancing the probability of disease, and the pretest probability would be high. 16

Table No 4: Advantages and Disadvantages of Biomarkers

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective assessment</td>
<td>Timing is critical</td>
</tr>
<tr>
<td>Precision of measurement</td>
<td>Expensive (cost for analysis)</td>
</tr>
<tr>
<td>Reliable; validity can be established</td>
<td>Storage (longevity of samples)</td>
</tr>
<tr>
<td>Less biased than questionnaires</td>
<td>Laboratory errors</td>
</tr>
<tr>
<td>Disease mechanisms often studied</td>
<td>Normal range difficult to establish</td>
</tr>
<tr>
<td>Homogeneity of risk or disease</td>
<td>Ethical responsibility</td>
</tr>
</tbody>
</table>

FUTURE DIRECTION

A large concerted effort is required to get advanced in the field of biomarker discovery. Most current biomarkers do not satisfy the required characteristic of use among the spectrum of diseases. Validation of a new biomarker is necessary. The generation of prospective data will demonstrate clinical utility. High throughput technology has begun to disease processes and biology detail and this would offer the potential to identify and characterize the novel biomarkers. Molecular biology is now seen as encouraging more in ‘Personalized medicine’ closer alignment of biological information and also for therapy selection. A well-designed effort will be needed to develop general knowledge about the molecular history of diseases to keep up with the progress with biomarker development. The evolution of molecular medicine, coupled with the discovery and clinical application of new biomarkers, will play a significant key role in reshaping or modifying medicine as a science.

Science in India could make a significant impact on the global science of the scientists and policymakers could agree to dedicate sufficient time and resources to the field of biomarkers. This should be much beyond the task force and excellence initiative and should be output drivers in a defined time.
SUMMARY

Many studies using biomarkers never achieve their full potential because of the failure to adhere to the same rules that would apply for the use of variables that are not biological. The development of any biomarker should precede or to go in parallel with the standard design of any epidemiological project or clinical trial. Informing the laboratory component, pilot studies must be completed to determine accuracy, reliability, interpretability, and feasibility. The investigator must establish “normal” distributions by important variables such as age and gender. The investigator will also want to establish the extent of intraindividual variation, tissue localization, and persistence of the biomarker. Moreover, he or she will need to determine the extent of inter-individual variation attributable to acquired or genetic susceptibility. Most, if not all of these issues can be resolved in the case of pilot studies preceding the formal investigation.

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REFERENCES