Review Article

A REVIEW ON 'BIOMARKERS' AS DIAGNOSTIC TOOL

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ABSTRACT

Biomarker is a biochemical feature or facet that can be used to measure the progress of disease or the effects of treatment. A biomarker can be a substance that is introduced into an organism as a means to examine organ function or other aspects of health. Biomarkers provide a dynamic and powerful approach to understanding the spectrum of neurological disease with applications in observational and analytic epidemiology, randomized clinical trials, screening and diagnosis and prognosis. These markers offer the means for homogeneous classification of a disease and risk factors, and the can extend our base information about the underlying pathogenesis of disease. Biomarkers can also reflect the entire spectrum of disease from the earliest manifestations to the terminal stages. This brief review describes the major uses of biomarkers in clinical investigation. Issues that affect the analysis of biomarkers are discussed along with recommendations on how to deal with bias and confounding.

Keywords: Antecedent biomarkers, diagnostic biomarkers, cardiac biomarkers, cancer biomarkers.

INTRODUCTION

Biological markers (biomarkers) have been defined by 'Hulka and colleagues[1],as "cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids." More recently, the definition has been broadened to include biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. In practice, biomarkers include tools and technologies that can aid in understanding the prediction, cause, diagnosis, progression, regression, or outcome of treatment of disease.

Biomarkers of all types have been used by generations of epidemiologists, physicians, and scientists to study human disease. The application of biomarkers in the diagnosis and management of cardiovascular disease, infections, immunological and genetic disorders, and cancer are well known[1,2,3]. Their use in research has grown out of the need to have a more direct measurement of exposures in the causal pathway of disease that is free from recall bias, and that can also have the potential of providing information on the absorption and metabolism of the exposures[4].

The rapid growth of molecular biology and laboratory technology has expanded to the point at which the application of technically advanced biomarkers will soon become even more feasible. Molecular biomarkers will, in the hands of clinical investigators, provide a dynamic and powerful approach to understanding the spectrum of neurological disease with obvious applications in analytic epidemiology, clinical trials and disease prevention, diagnosis, and disease management.[5,6,7,8]

TYPES OF BIOMARKERS

Biomarkers have been classified by Perera and Weinsteinbased[3] on the sequence of events from exposure to disease. Schulte[9] has outlined the capabilities of biomarkers. Biomarkers have the potential to identify the earliest events in the natural history, reducing the degree of misclassification of both disease and exposure, opening a window to potential mechanisms related to the disease pathogenesis, accounting for some of the variability and effect modification of risk prediction, these can also provide insight into disease progression, prognosis, and response to therapy. There are two major types of biomarkers:

1. Biomarkers of exposure, which are used in risk prediction, and
2. Biomarkers of disease; for screening, diagnosis, monitoring of disease progression.

In epidemiological (or quasi-experimental) investigations, biomarkers improve validity while reducing bias in the measurement of exposures (or risk factors) for neurological disease. Rather than relying on a history of exposure to a putative risk factor, direct measurement of the level of exposure or the chromosomal alteration resulting from the exposure lessens the possibility of misclassification of exposure.

Molecular biomarkers have the additional potential to identify individuals susceptible to disease. Molecular genetics have already had an impact on neurological practice, leading to improved diagnosis. Classification of populations in terms of the degree of susceptibility on the basis of such biomarkers produces greater accuracy than relying on historical definitions of susceptibility. For example, a biomarker will allow the stratification of a population on the basis of a specific "genotype"

associated with a disease rather than relying on a report of the "family history" of the disease.[10,11]

BIOMARKERS OF EXPOSURE OR ANTECEDENT BIOMARKERS

Environmental exposures, effect modifiers, or risk factors

External exposure is the measured concentration of the toxin in an individual's immediate environment. While questionnaires offer an historical account of the exposure, direct measurement of the alleged toxin in the air, water, soil, or food can provide accurate information regarding the "dose" of the exposure.

When the 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. The pharmacokinetic properties of the toxin or chemical become important to consider in measurement of the internal dose because a number of body fluids could be used based on the pharmacologic properties of the agent. Some chemicals such as halogenated hydrocarbons are stored in adipose tissue but others, such as organophosphate pesticides, are better measured in blood or urine.

Genetic susceptibility

Epidemiologic analyses can examine familial aggregation and assess genetic and environmental contributions to a disease by using life table methods and recurrence risk. Mutations in genes that result in Mendelian forms of disease are typically deterministic. Variant alleles in genes or polymorphisms may be related to susceptibility but are not deterministic. Most adult-onset degenerative diseases of the nervous system are likely to be a composite of related characteristics, heritable and environmental. The correlated combinations of these features constitute the trait or disease.

For neurological disorders, biomarkers of genetic susceptibility are rapidly becoming more available. Identification of the variant allele in a gene, such as *APOE* (apolipoprotein E), is quite useful in assessing risk and in providing information regarding the pathogenesis of the Alzheimer's disease. With this information investigators can examine other genes or environmental risk factors to determine whether they modified (increase or decrease) the risk of Alzheimer's disease or not. Similarly, variations in several genes appear to influence susceptibility to Parkinson's disease, which has also been related to environmental risk factors. Once established, a specific genotype might be used to predict an association with a particular environmental toxin[12].

Intermediate biomarkers

Some biomarkers represent direct steps in the causal pathway of a disease and are therefore strongly related to disease, others are related in some indirect way. There are numerous possibilities to consider. A biomarker could be dependent on another known or unknown factor to cause disease. Thus, it is not the only determinant but it is in the causal pathway and remains strongly related to the disease. The biomarker could also be related to an exposure that has already been identified or represents an alteration caused by the exposure that results in the disease. The most precarious situation is one in which the biomarker is related to some unknown factor that is also related to the exposure. This type of confounder, if unidentified, can decrease the validity of the association between the biomarker and the disease.

BIOMARKERS OF DISEASE

Biomarkers depicting prodromal signs enable earlier diagnosis or allow for the outcome of interest to be determined at a more primitive stage of 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 a subclinical manifestation, stage of the disorder, or a surrogate manifestation of the disease. Biomarkers used for screening or diagnosis also often represent surrogate manifestations of the disease. The potential uses of this class of biomarkers include:

1. Identification of individuals destined to become affected or who are in the "preclinical" stages of the illness,
2. Reduction in disease heterogeneity in clinical trials or epidemiologic studies,
3. Reflection of the natural history of disease encompassing the phases of induction, latency and detection, and
4. Target for a clinical trial.

Cardiac Biomarkers

Cardiac biomarkers are substances that are released into the blood when the heart is damaged. Measurement of these biomarkers is used to help diagnose, evaluate, and monitor patients with suspected acute coronary syndrome (ACS)[13]. The symptoms of ACS are associated with heart attacks and angina, but they may also be seen with non-heart-related conditions. Increases in one or more cardiac biomarkers can identify patients with ACS, allowing rapid diagnosis and appropriate treatment of their condition.

Cardiac biomarker tests are ordered to help detect the presence of ACS and to evaluate its severity as soon as possible so that appropriate therapy can be initiated. It is important to distinguish heart attack from angina, heart failure, or another condition because the treatments and monitoring requirements are different. For heart attacks, prompt medical intervention is crucial to minimize heart damage and future complications[14]. Cardiac biomarker tests must be available to the doctor 24 hours a day, 7 days a week with a rapid turn-around-time[15]. Some of the

tests may be performed at the point of care (POC) – in the Emergency Room or at the patient's bedside. Serial testing of one or more cardiac biomarkers is often done to ensure that a rise in their blood levels is not missed and to estimate the severity of a heart attack[16,17].

The current biomarker test of choice for detecting heart damage is troponin. Other cardiac biomarkers are less specific for the heart and may be elevated in skeletal muscle injury, liver disease, or kidney disease. Many other potential cardiac biomarkers are being researched, but their clinical utility has yet to be established[18,19].

Cancer Biomarkers

Cancer biomarkers are employed across the entire healthcare spectrum from the cancer biological research laboratory to patient monitoring in the clinic. Cancer biomarker's applications include the identification of novel therapeutic targets in cancer drug discovery and uses of cancer biomarkers as surrogate markers for drug efficacy in clinical trials. This report describes a number of factors providing the driving forces behind cancer biomarker growth and commercialization[20,21]. Emerging cancer biomarker types and the increasing interest in circulating tumour cells, as well as data on potential DNA, RNA, and protein biomarkers under study, includes Oncogenes, Germline inheritance, Mutations in drug targets, Epigenetic changes.

Biomarkers in Drug Development

With the increasing cost and complexity of drug development, biomarkers play an increasing role in the early phases of drug development. Biomarkers can be classified 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 any competitor drugs. Clinical validation of the biomarker has a direct influence on the clinical utility and therefore on the label of the co-developed product. As shown in Figure 1.

Table 1: COMMONLY USED CARDIAC BIOMARKER TESTS

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

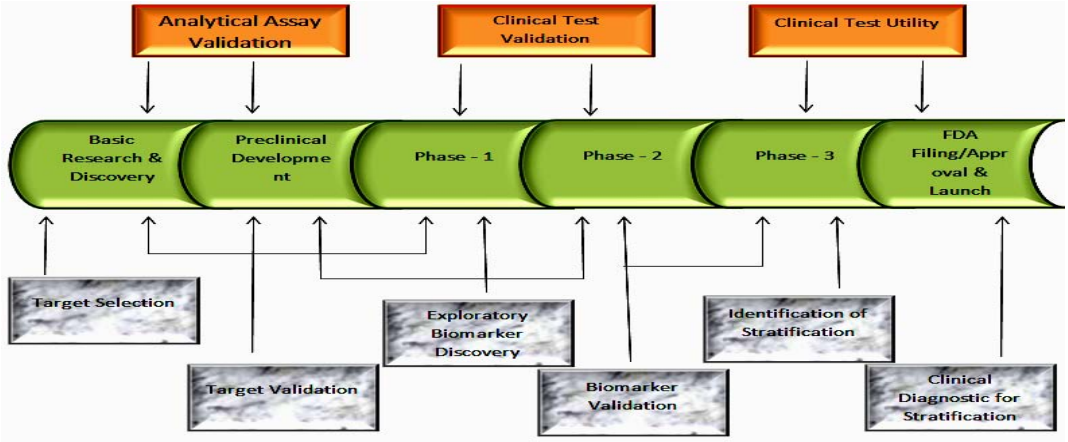
These tests are used to help diagnose, evaluate, and monitor patients suspected of having Acute Coronary Syndrome (ACS).

Table 2: Biomarker Tests User for Prognosis

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

These tests may be used to evaluate risk of future cardiac events.

Figure 1: Flow chart showing the importance of biomarkers in drug development process



VARIABILITY

Although 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 from the way a putative toxin is metabolized. For example, individuals exposed to the same chemical might differ in their ability (or inability) to metabolize the agent, or they may have experienced different types of exposures (in the field as compared with in the office). Intra-individual variability is usually related to laboratory errors or other conditions, or exposures unique to the individual. Group variability is also encountered, but this is often the desired outcome of a study. While measurement error is always a concern with biomarkers, other important factors may explain individual or group variability. Some workers may always wear protective equipment whereas others may not. Interaction with other exposures, drugs, or effect modifiers can increase or decrease the effect of the biomarker under consideration as an exposure or as a measure of susceptibility. Variability can also be attributed to the effects of factors such as individual diet or other personal characteristics. Amount of dietary fat can influence the biological measurement of lipid-soluble vitamins as well as toxic chemicals.

VALIDITY

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 all affect the reliability of the biomarkers used in any investigation. Kappa statistics for binary or dichotomous data and intra-class correlation coefficients should be used to assess test-retest agreement and consistency.

The evaluation of the validity of a biomarker is complex. Schulte and Perera[22] suggest three aspects of measurement validity:

1) Content validity, shows degree to which biomarker reflects bio-phenomenon studied,

2) Construct validity, which pertains to other relevant characteristics of disease or trait, and

3) Criterion validity, which shows the extent to which the biomarker correlates with the specific disease and is usually measured by sensitivity, specificity, and predictive power.[23,24]

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 actually have the disease. This provides us with information about the likelihood of the disease being present if the test is positive. Negative predictive value (NPV) is the percentage of people with a negative test who do not have the disease. Increasing the prior probability will increase the PPV but decrease the NPV, assuming that the sensitivity and specificity remain unchanged.

CONCLUSION

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 go in parallel with the standard design of any epidemiological project or clinical trial. In forming 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 intra-individual 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 pilot studies preceding the formal investigation.

REFERENCES

1. Hulka BS. Overview of biological markers. Biological markers in epidemiology, New York: Oxford University Press, 1990; 3-15.
2. Naylor S. Biomarkers: current perspectives and future prospects. Expert Rev MolDiagn 2003; 3: 525–529.
3. Perera FP, Weinstein IB. Molecular epidemiology: recent advances and future directions.Carcinogenesis 21: 2000: 517–524.
4. Gordis L. Epidemiology and public policy. Epidemiology. Philadelphia: W.B. Saunders, 1996: 247-256.
5. Verbeek MM, De Jong D, Kremer HP. Brain-specific proteins in cerebrospinal fluid for the diagnosis of neurodegenerative diseases. Ann J ClinBiochem 2003; 40: 25–40.
6. Galasko D. New approaches to diagnose and treat Alzheimer's disease: a glimpse of the future.ClinGeriatr Med 2001; 17: 393–410.
7. Rohlff C. Proteomics in neuropsychiatric disorders. Int. J Neuropsychopharmacol 2001; 4: 93–102.
8. Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J NeurolSci 2001; 184: 101–122.
9. Schulte PA. A conceptual and historical framework for molecular epidemiology. San Diego: Academic Press, 1993: 3-44.
10. Merikangas K. Genetic epidemiology: bringing genetics to the population-the NAPE Lecture 2001.Acta PsychiatrScand 2002; 105: 3–13.
11. Muller U, Graeber MB. Neurogenetic diseases: molecular diagnosis and therapeutic approaches. J Mol Med 1996; 74: 71–84.
12. Mayeux R. Epidemiology of neuro degeneration. Annu Rev Neurosci 2003; 26: 81–104.
13. Christenson, R., Editor (© 2007). Biomarkers of Acute Coronary Syndrome and Heart Failure. National Academy of Clinical Biochemistry, Laboratory Medicine Practice Guidelines. Available online at http://www.aacc.org/ AACC/ members/nacb/LMPG/OnlineGuide/ Published Guidelines/ACSHeart
14. Weinrauch, L. (2007 March 30, Updated). Heart Attack. MedlinePlus Medical Encyclopedia. Available online at http://www.nlm.nih.gov/medlineplus/ ency/article/000195.htm .
15. (2007 May). What is a Heart Attack. National Heart Lung and Blood Institute. Available online at http://www.nhlbi.nih.gov/health/dci/Diseases/HeartAttack/HeartAttack.html.
16. Schreiber, D. and Miller, S. (2006 June 26, Updated). Use of Cardiac Markers in the Emergency Department. emedicine. Available online at http://www.emedicine.com/emerg/TOPIC932.HTM.
17. Clinical Laboratory News: New NACB Guidelines Emphasize Cardiac Troponin for ACS. *Clinical Laboratory News* April 2007: V33 (4).
18. Pagana, Kathleen D. & Pagana, Timothy J. Mosby's Diagnostic and Laboratory Test Reference; 8th Edition: Mosby, Inc., Saint Louis, MO.2007; 319, 321, 668-670.
19. Clarke, W. and Dufour, D. R., Editors. Contemporary Practice in Clinical Chemistry, AACC Press, Washington, DC.2006; 306-315.
20. Bouras, T., Southey, M. C, and Venter, D. J. Overexpression of the steroid receptor coactivator AIB 1 in breast cancer correlates with the absence of estrogen and progesterone receptors and positivity for p53 and HER2/neu. Cancer Res,2001; 61: 903-907.
21. Cloven, N. G., Kyshtoobayeva, A., Burger, R. A., Yu, I. R., and Fruehauf, J. P. In vitro chemoresistance and biomarker profiles are unique for histologic subtypes of epithelial ovarian cancer. GynecolOncol, 2004; 92: 160-166.
22. Schulte PA, Perera FP. Validation. In: Molecular epidemiology: principles and practices (Schulte PA, Perera FP, eds),. San Diego: Academic Press, 1993: 79–107.
23. Pepe MS, Thompson ML. Combining diagnostic test results to increase accuracy. Biostatistics 2000; 1: 123–140.
24. Thompson ML, Zucchini W. On the statistical analysis of ROC curves. Stat Med 1989; 8:1277–1290

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