

WHAT IS A BIOMARKER? FROM ITS DISCOVERY TO CLINICAL APPLICATION ŠTA JE BIOMARKER? OD OTKRIĆA DO KLINIČKE PRIMENE

Nada Majkić-Singh

*Institute for Medical Biochemistry, Pharmaceutical Faculty and Clinical Centre of Serbia,
University of Belgrade, Belgrade, Serbia*

Summary: The term biomarker in medicine most often stands for a protein measured in the circulation (blood) whose concentration indicates a normal or a pathological response of the organism, as well as a pharmacological response to the applied therapy. From a wider perspective, a biomarker is any indicator that is used as an index of the intensity of a disease or other physiological state in the organism. This means that biomarkers have a very important role in medical research and practice providing insight into the mechanism and course of a disease. Since a large number of biomarkers exist today that are used for different purposes, they have been classified into: 1) antecedent biomarkers, indicating risk of disease occurrence, 2) screening biomarkers, used to determine a subclinical form of disease, 3) diagnostic biomarkers, revealing an existing disease, 4) staging biomarkers, that define the stage and severity of a disease, and 5) prognostic biomarkers, that confirm the course of disease, including treatment response. Regardless of their role, their clinical significance depends on their sensitivity, specificity, predictive value, and also precision, reliability, reproducibility, and the possibility of easy and wide application. For a biomarker to become successful, it must undergo the process of validation, depending on the level of use. It is very important for every suggested biomarker, according to its purpose or its nature, to possess certain characteristics and to meet the strict requirements related to sensitivity, accuracy and precision, in order for the proper outcome to be produced in the estimation of the state for which it is intended. Finally, the development of guidelines for biomarker application is very important, based on well defined and properly conducted assessments of biomarker determination, providing the means by which research is translated into practice and allowing evidence based on facts to promote the clinical application of new biomarkers.

Keywords: biomarkers, definition, discovery, clinical significance

Kratak sadržaj: Izrazom biomarker u medicini se označava najčešće protein izmeren u cirkulaciji (krvi) čija koncentracija ukazuje na normalan ili patološki odgovor u organizmu, kao i na farmakološki odgovor na primenjenu terapiju. Šire gledano biomarker je bilo koji pokazatelj koji se koristi kako indikator intenziteta nekog oboljenja ili drugog fiziološkog stanja u organizmu. To znači da biomarkeri imaju veoma značajnu ulogu u medicinskim istraživanjima i primeni jer omogućavaju sagledavanje mehanizma i toka bolesti. Pošto danas postoji veliku broj biomarkera koji se koriste u različite svrhe izvršena je njihova podela na: 1) antecedentne biomarkera, koji ukazuju na rizik od nastanka bolesti, 2) »screening« biomarkere, kojima se utvrđuje subklinička forma bolesti, 3) dijagnostičke biomarkere, koji otkrivaju postojeću bolest, 4) »staging« biomarkere, koji definišu stadijum i težinu bolesti i 5) prognostičke biomarkere, koji potvrđuju tok bolesti, uključujući i odgovor na terapiju. Bez obzira na ulogu koju imaju, njihov klinički značaj zavisi od njihove osetljivosti, specifičnosti, prediktivne vrednosti, zatim preciznosti, pouzdanosti, reproduciбилности, kao i mogućnosti jednostavne i široke primene. Da bi biomarker bio uspešan mora proći kroz put validacije zavisno od nivoa upotrebe. Veoma je značajno da svaki predloženi biomarker zavisno od namene ili njegove prirode ima odgovarajuće karakteristike i da ispunjava stroge zahteve koji se odnose na osetljivost, tačnost i preciznost kako bi se postigao odgovarajući ishod za procenu stanja za koji je namenjen. Shodno navedenom više istraživačkih cenatara i grupa predložilo je način i uputstva za evaluaciju biomarkera uzimajući u obzir prognostičke prema dijagnostičkim modelima. Konačno, veoma je značajna izrada uputstava (Guidelines) za primenu biomarkera koja su zasnovana na dobro definisanim i sprovedenim procenama određivanja nekog biomarkera, na koji način su dobijena dobra sredstva putem kojih su preneti istraživanja u praksu a dokazi zasnovani na činjenicama potpomažu kliničku primenu novih biomarkera.

Ključne reči: biomarker, definicija, otkriće, klinički značaj

Address for correspondence:

Prof. dr Nada Majkić-Singh
Institute for Medical Biochemistry
Pharmaceutical Faculty and Clinical Centre of Serbia
University of Belgrade, Belgrade, Serbia
e-mail: singh@eunet.rs

Introduction

The term biomarker in medicine most often stands for a protein measured in the circulation (blood) whose concentration indicates a normal or a pathological response of the organism, as well as a pharmacological response to the applied therapy. From a wider perspective, a biomarker is any indicator that is used as an index of the intensity of a disease or other physiological state in the organism. This means that biomarkers have a very important role in medical research and practice providing insight into the mechanism and course of a disease.

A biomarker can also be a substance that when introduced into the organism serves for the estimation of organ function or some other form of health assessment. For instance, rubidium chloride is used as a radioactive isotope to estimate cardiac muscle perfusion. A biomarker can further be a substance used to discover the presence of antibodies indicating infection. More often, biomarkers indicate changes in the expression of a protein that is correlated to risk or progression of a disease or its response to treatment, and can be measured e.g. in tissues or in the blood. This means that biomarkers can be specific cells, molecules or genes, gene products, enzymes or hormones. Biomarkers may be used to characterize complex organ functions or typical general alterations in biological structures. Although the term itself has been introduced recently, biomarkers have been used in preclinical research and clinical diagnostics for a long time. For instance, body temperature has been used for years as a biomarker of fever; blood pressure is a marker of the risk of stroke, and cholesterol is both a marker and risk factor for coronary and vascular diseases, while C-reactive protein is a marker of inflammation.

Since a large number of biomarkers exist today that are used for different purposes, they have been classified into: 1) antecedent biomarkers, indicating risk of disease occurrence, 2) screening biomarkers, used to determine a subclinical form of disease, 3) diagnostic biomarkers, revealing an existing disease, 4) staging biomarkers, that define the stage and severity of a disease, and 5) prognostic biomarkers, that confirm the course of disease, including treatment response (1).

Regardless of their role, their clinical significance depends on their sensitivity, specificity, predictive value, and also precision, reliability, reproducibility, and the possibility of easy and wide application (2). The most significant biomarkers are those that form an integral part of diagnostic and prognostic algorithms, such as natriuretic peptides in cardiac insufficiency (3). In addition, special significance is attributed today to the biomarkers whose level changes following the application of certain treatment, especially when this change correlates with the outcome

(4). It is therefore necessary to prove that the applied treatment and outcome change significantly depending on whether or not the prognostic and therapeutic algorithm includes the observed biomarker, which is the domain of prospective clinical studies (4, 5).

New tests are being introduced every day, and the technology of the existing ones is constantly improving. The assessment of a diagnostic test should thus contribute not only to its introduction into clinical practice, but also to the reduction of unwanted clinical consequences related to the accuracy of the test itself, and thereby to the reduction of the costs of unnecessary repetition of laboratory determinations. Diagnostic test assessment is an intricate process, even though clinical accuracy and diagnostic accuracy are the two most important factors in this assessment. This fact has already been acknowledged, especially in the procedure of randomly done clinical trials, which has led to the *Consolidated Standards of Reporting Trials – CONSORT* statement, providing a list of questions to be answered during the assessment of diagnostic accuracy.

Standards for the Reporting of Diagnostic Accuracy – STARD, describe all the necessary evidence needed for meeting certain requirements listed in the *CONSORT* statement (6, 7). This provides a platform for deliberate application of the concept of *Evidence-Based Laboratory Medicine – EBLM*, and for a number of reasons (8, 9). First, it is necessary to ensure the best possible results, thus enabling physicians to make diagnostic, prognostic and therapeutic decisions. On the other hand, it is necessary to assess a larger number of diagnostic tests. Thirdly, the question arises of how efficient the diagnostic tests are and what is their price (10).

For the reasons given above, the symposium entitled »Biomarkers: from Standardization to Performance« will be dealing with answers to the following issues: the question what are biomarkers and what is their clinical application, standardization procedures and defining the feasibility and clinical validity of a standard, presenting examples and the importance of specific standards in cardiovascular diseases (e.g. apolipoproteins B and A, copeptin, myeloperoxidase etc.), diabetes and its complications (e.g. products of non-enzymatic posttranslational modification, HbA1c, TIV collagen, hepatocyte growth factor etc.), biomarkers of bone turnover, tumor markers and biomarkers of fetal anomalies (11–15).

From discovery to definition

The fact that the National Institutes of Health in the USA financed in the period from 1986 to 2009 around 30 000 projects related to biomarker research (or only containing the term biomarker) highlights the importance of this issue. The total invested sum

reached around 3 billion dollars only in 2008 and 2009. Searching PubMed for the term »biomarker« between 1990 and 2010 would reveal over half a million published articles (16). In view of this expansion of research and publications related to biomarkers, as well as the diverse terminology in the literature, the NIH formed the Biomarker Definition Working Group – BDW, with the aim of elucidating the growing confusion and suggesting a specific definition for biomarkers. In 2001 this group suggested specific definitions for clinical and surrogate biomarkers (3). According to their definition, a biomarker is a characteristic objectively measured and estimated as an indicator of normal biological processes, pathological processes or a pharmacological response to therapeutic intervention. Clinical response – the clinical end-point, is a characteristic or an alteration that reflects the patient's state, functioning or survival. Surrogate end-point is a biomarker that should replace the clinical end-point, i.e. that is fully alternative to the one used to estimate the clinical end-point (17). Such a biomarker is expected to predict clinical benefit (harm or lack of it) according to the epidemiologic, therapeutic, pathophysiological or other evidence using which it is possible to achieve prediction of benefit. Conclusion may be drawn from the above definition that biomarkers can be numerous instruments serving as prognostic or diagnostic indicators of disease or sensitive and specific tools for risk assessment. Biomarkers can be biological, physical or molecular in their nature.

With reference to the above, biomarkers can be classified depending on parameters. For instance, depending on their characteristics, they can be classified as imaging biomarkers (CT, PET, MRRI) or molecular biomarkers. Molecular biomarkers are measured on the basis of biophysical properties in biologic samples (e.g. plasma, serum, cerebrospinal fluid, bronchoalveolar lavage, biopsy), and may refer to nucleic acids biomarkers, such as gene mutations or polymorphisms, peptides, proteins, lipid metabolites and other small molecules.

Depending on their application, biomarkers can also be classified into:

- 1) diagnostic biomarkers (e.g. cardiac troponins for diagnosing myocardial infarction),
- 2) biomarkers to determine the stage of disease (e.g. brain natriuretic peptide for determining cardiac insufficiency),
- 3) prognostic biomarkers (tumor markers),
- 4) biomarkers for monitoring clinical response (e.g. HbA1c for antidiabetic treatment).

Another group are biomarkers determined in the development and clinical investigation of drugs. Pharmacodynamic biomarkers are markers of the

pharmacological response and have particular significance in drug dosage optimization.

Based on genetic and molecular biologic methods, biomarkers are classified into three types:

Type 0 – Natural history markers (prognosis)

Type 1 – Biological activity markers (response to therapy)

Type 2 – Surrogate markers (single or multiple markers of therapeutic efficacy).

It is necessary to make a distinction between *disease-related* and *drug-related biomarkers*. Disease-related biomarkers point to whether a disease should be treated (risk indicator or predictive biomarker), whether a disease already exists (diagnostic biomarker), or whether a disease will continue to develop (prognostic biomarker). Contrary to this, drug-related biomarkers indicate whether a drug will be effective in a specific patient and how the organism will tolerate it. Today, biomarkers are also the key to personalized medicine, i.e. treatment that is applied to each patient individually.

A classic biomarker in medicine is a laboratory parameter that enables a physician to reach a diagnosis and choose proper treatment. For instance, a finding of certain autoantibodies in the blood of a patient is a reliable marker of autoimmune disease. A finding of rheumatoid factors has been used for over fifty years as a marker of rheumatoid arthritis. Today it is known that the appearance of ACPA (anti-citrullinated protein/peptide antibody) in the blood may indicate rheumatoid arthritis even before symptoms occur. This biomarker is therefore of utmost value as a predictive biomarker for early diagnosis of this autoimmune disease. The same biomarker may also be useful for monitoring the efficacy of RA treatment.

In the past biomarkers have primarily been physiologic indicators (e.g. blood pressure or pulse). Today many examples suggest that biomarker has become a synonym for a molecular biomarker (e.g. first of all enzymes for the estimation of diseases of the liver, heart and other organs) or prostate-specific antigen as a marker of prostate cancer. There are numerous other examples and other tumor markers in oncology.

Molecular biomarkers are also used in the initial stages of drug development. In the first stage of research, they are used to establish the necessary doses for the 2nd research stage. Tests for estimating the function of liver (e.g. transaminases, bilirubin, alkaline phosphatase), kidney (creatinine, creatinine clearance, cystatin C), skeletal muscles (myoglobin), cardiac muscle (CK-MB, troponins) etc. are used both in the preclinical and clinical investigation of a drug.

Contemporary methods for the discovery of a molecular biomarker

If a biomarker is to be used for diagnosing disease, the material where it is determined must be easily obtained. It can be a sample of blood, urine, saliva, or some other biologic material. The method being used for the determination must be accurate and easily performed. Results should preferably be obtained quickly and reveal no inter-laboratory differences, and the biomarker must be used effectively for the diagnosis, prognosis or risk estimation in a patient.

Many biomarkers are being used in laboratory medicine today, for whose determination numerous methods are used, from classic to molecular. As a consequence, various expressions are used to signify the ways of determining biomarkers, like for example Metabolomics, Lipidomics, Glycomics etc. The expression Metabolomics (or Metabonomics) globally signifies the analysis of all metabolites in biologic samples. Lipidomics stands for the analysis of lipids and lipid metabolites by numerous techniques such as mass spectrometry, chromatography, nuclear magnetic resonance, etc. The Genomic Approach includes the following techniques: Northern blot, gene expression, SAGE, DNA Microarray (18), while the Proteomic Approach implies: 2D-PAGE, LS/MS, SELDI-TOF, Ab Microarray, Tissue Microarray.

Depending on the way information are obtained, three types of biomarkers may be distinguished: 1) biochemical or histological parameters that are detected in tissue samples obtained by biopsy or surgery, 2) biochemical parameters or cells obtained from blood or urine samples, and 3) anatomic, functional or molecular parameters detected through the use of imaging techniques (19). The coupling of biomedical imaging techniques and e.g. biochemical markers is especially recommended for the early diagnosis of carcinoma, as well as for further promotion of the diagnostic and therapeutic strategy.

Many novel biomarkers have been developed that use the imaging technology. Their advantage is that they are usually non-invasive, and are characterized by both qualitative and quantitative multidimensional results. They are comfortable for patients and in combination with other information they are useful to clinicians for establishing a diagnosis. Great hopes are placed especially in the development of imaging techniques in cardiology and cardio-computed tomography (CT). Today it is possible to diagnose benign and malignant diseases by using ultrasound (US), computerized tomography (CT) and magnetic resonance imaging (MRI) on the basis of discovering morphological alterations in the organism. These techniques allow for the study of morphological alterations and functional biological pathways and disturbances (20). Contemporary imaging biomarkers are largely based on nuclear imaging technologies

such as scintigraphy, single-photon emission tomography (SPET) and positron emission tomography (PET) (21).

For a biomarker to become successful, it must undergo the process of validation, depending on the level of use. It is very important for every suggested biomarker, according to its purpose or its nature, to possess certain characteristics and to meet the strict requirements related to sensitivity, accuracy and precision, in order for the proper outcome to be produced in the estimation of the state for which it is intended. Hence, several research centers and groups have recommended the means and guidelines for biomarker evaluation considering prognostic as opposed to diagnostic models (22–24).

The development path of every biomarker from its discovery in a laboratory to its inclusion into clinical practice includes the following five stages:

1. preclinical investigation in the developmental laboratory, where the method is applied to different biological materials, e.g. nuclear cells, blood, urine, saliva, tissue, in which the target biomarkers are identified – genes, proteins, enzymes and other substances; values of the investigated biomarker are then compared in healthy and diseased subjects in order to establish the extent of their correlation with the investigated biological phenomena (e.g. serum CRP reflects systemic inflammation); after this the very method is perfected, i.e. its reliability and sensitivity;
2. in the second stage the validity of the investigated biomarker is assessed, i.e. its ability to identify diseases compared to the gold standard, and the reference values are determined along with intra-individual variations;
3. stage 3 implies retrospective epidemiological studies on screening and the predictive value of the biomarkers;
4. stage 4 includes prospective clinical studies to investigate the correlation between biomarker levels and the onset of clinical indicators of the disease course;
5. stage 5 is where randomized clinical controlled studies are performed to assess whether the treatment modified by the application of the investigated biomarker is better in relation to the previous, and the influence of drugs on biomarker values is investigated.

Before introducing a biomarker into clinical practice it is also necessary to have answers to several important questions, namely: a) what is the distribution of the investigated biomarker in the general and the observed population, and if there are variations depending on the basic demographic characteristics such as gender, age and race, which may impose certain limitations on their applicability; b) whether

the investigated biomarker correlates with the known risk factors or reflects other pathophysiological mechanisms that participate in the investigated disease; c) what are the limits of biomarker values and what is their sensitivity and specificity, and d) does its inclusion increase the prognostic value of the existing prognostic models.

The discovery, qualification and development of new IVD assays is a painstakingly difficult, slow and risky job. Many companies are dealing with this issue, with the aim of detecting the best biomarkers. To this end it is first of all necessary to define the assignment of the biomarker in relation to the scientific knowledge and technical possibilities. This is followed by a series of experiments in which an initial hypothesis leads to a new marker for differential diagnosis of the supposed disease (e.g. myocardial infarction). A very important step in the discovery of a biomarker includes efforts to obtain and collect proper clinical samples, in accordance with the defined standard operative procedures (SOPs) in order to ensure that the collection and storage of samples do not cause e.g. interference with the measurement of the biomarker itself. It is further important to choose a proper methodological approach, that should allow the determination of thousands of samples, with adequate reliability, and also be relatively easy to use. The developed prototype of IVD determination is then tested in clinical practice, prior to becoming commercially available. The standardization of tests for the purpose of clinical and chemical determinations is done on the basis of international standards. Metrological principles are e.g. described in the standards of the International Organization for Standardization (ISO), mostly ISO/CEN 17511 and ISO 18153. Since 1998, EU Directive on In Vitro Diagnostic Medical Devices (IVD-MD) (Directive 98/79/EC) requires traceability of calibrators and control materials to reference measurement procedures and/or reference materials of higher order. This means that IVD manufacturers worldwide today must ensure that the systems they are putting on the market are correctly calibrated according to certified reference materials and referent measurement procedures. Traceability of measurement according to the above stated concept is based on a stable reference value that is in accord with the SI system.

The importance of the EBLM principle for biomarker development

Evidence-based laboratory medicine (EBLM) uses the best evidence gained in the form of laboratory determination results for reaching a decision on providing care for each individual patient. This

approach is possible only on the basis of compiling laboratory and clinical experiences about the ways of treatment stemming from systematic investigations in these fields. Evidence-based laboratory medicine therefore has the goal to support clinical diagnosis and manage diseases by applying new insights leading to a standard procedure that would provide the best laboratory examination.

Many challenges are being posed before laboratory medicine, starting first from the quality of the service provided, as well as its efficacy, all depending on the available conditions. Too many laboratory determinations are known to be performed today, with a limited role in the final improvement of the outcome in a patient (25, 26). This is why the demands have been set for defining evidence and clinically practical protocols depending on clinical practice (27, 28). To achieve this, it is necessary to search literature data and clinical investigations which provide the highest diagnostic accuracy. With the goal of providing valid evidence, the STARDT statement has been published (24), aiming to improve the diagnostic accuracy in investigations. The protocol for a test examination should reflect a minimum of the conditions for its purpose. For this reason, another document, the CONSORT document (29, 30), provides methodology and guidelines for estimating randomly performed examinations. In order for the published data to be used in the best possible way, a list of characteristics which have to be fulfilled during the gathering of data with application examples has been made (24). The checklist elements can be found on a few web pages, including the one by the Consort group (30). Through the application of the given principles, cardiac markers troponin I and T have been ranked as A/B degrees, i.e. AB/C, while myoglobin, CK and CK-MB have been ranked as C degree (31–34).

Finally, the development of guidelines for biomarker application is very important, based on well defined and properly conducted assessments of biomarker determination (35), providing the means by which research is translated into practice and allowing evidence based on facts to promote the clinical application of new biomarkers.

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Conflict of interest statement

The author stated that there are no conflicts of interest regarding the publication of this article.

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