Biomarkers in Modern Healthcare: Types, Detection Methods, and Their Role in Transforming Patient Care

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Abstract

Biomarkers are measurable indicators of biological processes that play a critical role in early disease detection, prognosis, and informing treatment strategies. This literature review explores the various types of biomarkers, including molecular, physiological, radiographic, and histological, while emphasizing their clinical significance in diagnosing diseases such as cancer, cardiovascular disease, and neurodegenerative disorders. The review also compares widely used detection techniques, such as ELISA, chromatography, and mass spectrometry, with emphasis on their strengths and limitations. Emerging technologies like the digital ELISA and LC-MS are improving sensitivity and specificity, enhancing clinical utility at lower concentrations and greater accuracy. Despite the numerous advantages, such as non-invasive sampling and cost-effectiveness, challenges remain regarding consistent specificity across all biomarkers and translating surrogate endpoints into meaningful patient outcomes. Future innovations like AI-based analysis and genetic sequencing are expected to expand biomarker discovery and application. As research progresses, biomarkers are likely to become increasingly integrated into healthcare, offering powerful tools for improving diagnosis, treatment, and patient outcomes.

Introduction

Biomarkers have transformed the clinical field, acting as indirect medical indicators that assist in diagnosing diseases and predicting disease progression and outcomes [1]. Biomarkers can be found in blood, body fluids, or tissues, and they typically differentiate an infected patient from a healthy person [2]. Biomarkers are most commonly known for their role in detecting and diagnosing disease. A great example of a biomarker is the prostate-specific antigen (PSA), which facilitates the detection of prostate cancer. Unlike needing a biopsy, PSA detection makes diagnosing prostate cancer a less invasive process, more cost-effective, and ultimately more sensitive, and can lead to earlier detection (Figure 1). Additionally, a disease's advancement, progression, or regression requires measurable outcomes that are not overly impactful on a patient. Prognostic biomarkers, such as HER2 in breast cancer, allow for the measurement of disease progression [3]. More recently, biomarkers' role in drug discovery has become equally critical, serving as endpoints in clinical trials to help assess the safety and efficacy of new therapies [4]. Again, these biomarkers provide a less invasive and cost-effective method of determining the outcome of clinical trials. Due to their significant value in disease detection, prognosis, and outcomes, the discovery and detection of biomarkers have seen substantial advancements over the years, with many new and more sensitive technologies being developed specifically for biomarker detection. Over the past 30 years, the enzyme-linked immunosorbent assay (ELISA) has become a ubiquitous tool across various research fields for biomarker detection [5]. This literature review will examine the current findings in biomarker research, focusing on their diagnostic and clinical significance, as well as the various

bioanalytical methods, including ELISA, mass spectrometry, and chromatography-based techniques, that enable biomarker detection.

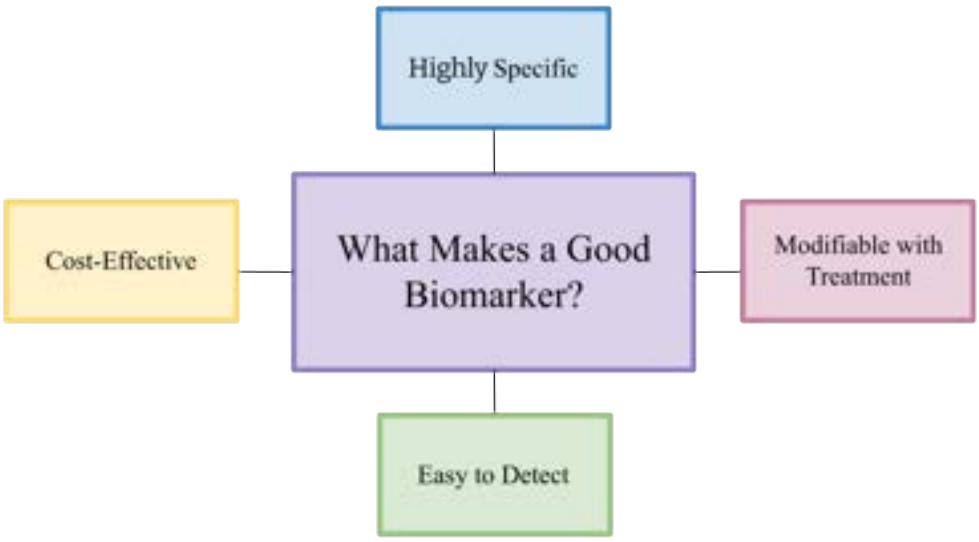

Figure 1. Elements of a Suitable Biomarker: Biomarkers provide many advantages over traditional clinical measurements as long as they can be easily detected, cost-effective, maintain specificity, and be able to measure changes in whichever outcome is being measured. [6]

Types of Biomarkers

Biomarkers can be classified into several types, including molecular, physiological, radiographic, and histologic biomarkers.

To begin, molecular biomarkers, measured based on a biomarker's molecular properties, offer a promising potential for early intervention [7]. They can detect subtle changes in biology at the molecular level before clinical symptoms are present, opening up new avenues for disease management and treatment [8].

Physiological biomarkers, used to measure the body's physiological or pathological status, are dynamic and changeable [9]. They offer actionable outcomes for prevention, assessment, and treatment, and their non-invasive, easily measurable nature makes them practical for ongoing evaluation, instilling confidence in their application [10]. Examples of physiological biomarkers include pulse and heart rate, blood pressure, and weight [11]. Radiographic biomarkers are markers derived from medical images that inform disease detection, characterization, and treatment response [12]. Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), and ultrasonography biomarkers are types of radiographic biomarkers that are used extensively in cancer research and drug development [13].

Finally, histological biomarkers are typically detected in cells and tissues from biopsies and tissue samples during surgery [14]. Histology enables the assessment of specific molecular markers and cell types within a tissue sample, such as a tumor, to better characterize the disease state and inform treatment decisions. With the expansion of research on the tumor

immune microenvironment and the successes and dissemination of immunotherapies for cancer treatment, pathologists are beginning to integrate these histologic and molecular features of the tumor microenvironment to provide the best prognostic information to clinicians and patients [15].

Biomarker Usage

Biomarkers serve a wide range of purposes, primarily serving as diagnostic, prognostic, and surrogate endpoint biomarkers. Diagnostic biomarkers are used to detect the presence of a specific disease, such as cardiac troponins, which can quickly identify myocardial infarction [16]. To accurately detect myocardial infarction, the detection of the particular isoform of the troponin complex, particularly troponin I (cTnI) and troponin T (cTnT), has to be distinguished from other isoforms present in different tissues, such as troponin C found in skeletal muscle [16]. More recently, iron has been identified as a significant diagnostic biomarker for detecting Alzheimer's disease. Disruptions in iron homeostasis, specifically the accumulation of excess iron in brain regions, contribute to the deposition of β-amyloid and the formation of neurofibrillary tangles, two features characteristic of Alzheimer's disease [17]. In this case, the amyloid plaques and tau aggregations serve as blood-based diagnostic biomarkers that link high levels of ferritin to cognitive impairment [18]. In contrast, the current diagnosis of Alzheimer's disease relies largely on documenting mental decline, at which point, Alzheimer's has already caused severe brain damage [19]. A recent study has shown that Aβ42/Aβ40 and pTau217 have shown the best diagnostic performances and seem to be the most promising candidates for analyses on at-risk' AD populations [20]. Although these are promising diagnostic markers for Alzheimer's, many undiscovered biomarkers could provide even more advanced identification of risk for the disease.

Biomarkers continue to play a crucial role in healthcare by monitoring disease progression. A prognostic biomarker, a clinical or biological characteristic that provides information on a patient's likely health outcome, is a key player in the era of personalized medicine [21]. For instance, in breast cancer, categorical combinations of immunohistochemical markers are commonly used to classify patients into subtypes, offering customized treatment strategies [22].

These classifications have direct implications for prognosis, treatment, and strategy. For example, hormone receptor statuses, specifically the estrogen receptor (ER) and progesterone receptor (PR), can help determine the efficacy of endocrine therapy, while HER2-positive patients identify candidates for targeted treatments, such as trastuzumab [23]. The ability to detect HER2 in breast cancer patients has led to a significant improvement in prognosis following HER2-targeted therapies. A meta-analysis of 13,864 women found that adding trastuzumab to chemotherapy reduced recurrence by 34% and mortality by 33% [24]. Finally, the Ki-67 proliferation index provides information about tumor aggressiveness, thereby offering valuable prognostic information that helps clinicians determine whether a patient may benefit from more intensive therapies [23]. Similarly, prognostic biomarkers also include BCR-ABL fusion genes in chronic myeloid leukemia, which are found in approximately 95% of CML patients due to the presence of the Philadelphia chromosome and can help inform disease treatment [25].

A surrogate endpoint is an endpoint that is used as a substitute for a direct clinical outcome measure of how a patient feels, functions, or survives [26]. While some clinical trials

recruit patients based on the presence of specific biomarkers and file for a companion diagnostics label, others examine these biomarkers for research use to enable internal decision-making and build clinic-genomic datasets [27]. By using these biomarkers to specifically recruit certain patients into clinical trials, it increases the chance of success for these therapies to be effective and ultimately reach approval, where they can then be used by clinicians to treat a broader population. Furthermore, these endpoints inform doctors on what patients will most likely respond to these therapies, making their use more effective. Surrogate endpoints are also used when the clinical outcomes might take a lengthy time to study, or in cases where the clinical benefit of improving the surrogate endpoint, such as controlling blood pressure, is well understood [1]. Studies with surrogate endpoints can be conducted rapidly and with less resource use and expense than endpoint studies [28]. In blood pressure, clinical trials have shown that reducing systolic blood pressure decreases the risk of major adverse cardiovascular events, including stroke, myocardial infarction, and cardiovascular death [29] [30]. The measurement of reduction in the surrogate endpoint of systolic blood pressure can thus serve as a substitute for the clinical outcome of the following cardiovascular events and can be conducted more rapidly in smaller populations using this validated surrogate endpoint [29] [30]. The use of biomarkers in surrogate endpoints helps accelerate the drug development process and informs treatment matching. To make use of biomarkers, accurate and reliable detection methods must be ensured.

Biomarker Detection Methods

Biomarker analysis involves identifying and verifying a specific gene or protein signature, which can serve as a quantifiable and well-defined indicator linking to a particular biological or clinical outcome [32]. Accurate biomarkers must have a high level of specificity. For example, as mentioned previously, cardiac troponin assays are designed to distinguish specific isomers, such as cTnT and cTnI, from skeletal forms; otherwise, false-positive and false-negative diagnoses can be reported [33]. The discovery and varied use of biomarkers will help ensure that patients receive treatment and medication using the best available therapeutic strategies, thereby minimizing unnecessary treatments and ultimately lowering total healthcare costs [34]. Accurate and sensitive biomarker detection methods are crucial for utilizing biological markers as reliable tools for clinical diagnostics and therapeutic monitoring.

Among the most widely used techniques is the enzyme-linked immunosorbent assay (ELISA), which detects significant antibodies, antigens, and other substances in your blood, urine, or other bodily fluids [35]. Initially, the conventional, analog ELISA required large volumes, which ultimately diluted the reaction product, using millions of enzyme labels to generate signals detectable with conventional plate readers [36]. Therefore, the sensitivity of traditional ELISA, limited to the picomolar range and above, is often insufficient for detecting specific biomarkers, especially those critical in diseases such as neurological disorders and cancer [37] [36]. Now, scientists and researchers are increasingly using digital ELISA. The development of the digital ELISA was designed to be highly efficient in capturing target proteins, labeling these proteins, and detecting them in single-molecule arrays [38]. The ELISA utilizes enzyme-labeled antigens and antibodies to detect the biological molecules, the most commonly used enzymes being alkaline phosphatase and glucose oxidase [39]. The most widely used ELISA assay format is the sandwich ELISA assay, which indirectly immobilizes and indirectly detects the presence of the target antigen [40, Figure 2]. The antigen binds to a specific antibody, which is then detected

by a secondary, enzyme-coupled antibody, resulting in a visible color change or fluorescence [39]. These biological markers can help diagnose a wide range of conditions, from bacterial and viral infections, such as Lyme disease and HIV, to endocrine disorders, including thyroid disease [35]. Despite its many advantages, the ELISA requires a tedious assay procedure and is limited to its known substances [41].

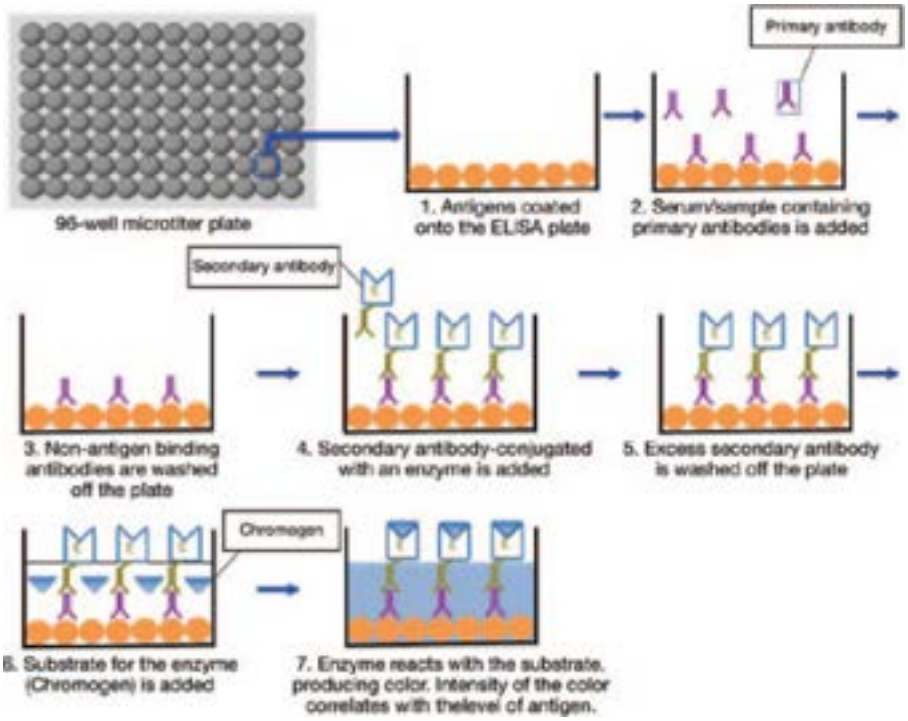

Figure 2. ELISA Technique: The diagram illustrates the general procedure for the ELISA. The process involves antigen coating, antibody binding, and colorimetric readout for quantifying results. [39]

A similar method is chromatography, specifically high-performance liquid chromatography, HPLC. The versatility of HPLC enables the precise separation, identification, and quantification of biomolecules, including proteins, lipids, hormones, and metabolites, making it a powerful tool for the discovery and monitoring of biomarkers [42][43]. This is particularly useful when other detection methods are not possible, such as detection by ELISA. It also allows for the detection of subtle molecular differences, such as drug metabolites, which other methods may not have the same level of specificity to detect. HPLC separates individual compounds from mixtures based on how they interact with a stationary phase and a mobile phase under high pressure [42].

In tandem with this, the biomarker detection method of mass spectrometry (MS) plays a significant role in biomarker discovery and detection. Mass spectrometry can precisely identify molecular structures and quantify their concentrations with precision and accuracy [44]. This technique focuses on identifying specific species in a sample by their atomic or molecular mass [45]. The technique can involve various protein expression techniques. Mass spectrometry has led to the discovery of thousands of potential biomarkers for cancer and other diseases [46]. MS-based quantitation techniques often rely on an initial digestion of the protein, and the subsequent protein quantitation is based on the quantitation of prototypic peptides that act as

surrogates for the protein of interest [46]. Thus, mass spectrometry can provide detailed molecular information, which is crucial for identifying biomarkers and developing targeted therapies [47]. MS-based proteomics and metabolomics have been particularly valuable in oncology, where the number and percentage of oncology clinical trials that include biomarkers have grown substantially: 55 percent of all oncology trials in 2018 involved the use of biomarkers, as compared with 15 percent in 2000, in turn facilitating the discovery of cancer-specific markers and potential therapeutic targets [48][47]. Additionally, the use of MS in therapeutic drug monitoring has enabled precise dosage adjustments based on individual pharmacokinetics and pharmacodynamics, significantly improving patient care by enhancing treatment efficacy and reducing adverse effects [47].

Method	What It Detects	Strengths	Limitation
ELISA	Proteins, antibodies, or hormones	Inexpensive, high-throughput, specific	Limited to known targets
Mass Spectrometry	Protein, metabolites, lipids	Highly sensitive, detects multiple targets	Expensive, complex sample prep
Chromatography	Metabolites, peptides, small molecules	Separates complex mixtures	Often needs to be combined with other methods like MS

Table 1. Comparison of Biomarker Detection Methods: Comparison of biomarker detection methods, ELISA, Mass Spectrometry, and Chromatography, based on what they detect, their key strengths, and their limitations. Each method plays a significant role in identifying proteins, metabolites, and small molecules within biological samples [31].

Another significant detection method that combines both liquid chromatography and mass spectrometry is liquid chromatography-tandem mass spectrometry, also known as liquid chromatography-mass spectrometry, or LC-MS. Its unique strength lies in its ability to separate, identify, and quantify the components of a complex biological sample by merging the physical separation of LC with MS's molecular identification and quantification abilities [44]. LC-MS is also prominent in detecting cerebrospinal fluid (CSF), with an accuracy of 83% and specificity of 100%, to identify altered protein pathways during neurodegenerative disorders, such as amyotrophic lateral sclerosis [49].

The following detection techniques, including ELISA, HPLC, MS, and LC-MS, have collectively contributed significantly to our ability to detect, monitor, and understand diseases with greater specificity. Although all of these methods are widely used in the biomarker field, each has its own strengths and limitations (Table 1). These methods are continuously being improved, and new technologies are continuing to be developed to more easily and cost-effectively detect biomarkers with the ultimate goal to reduce the burden on patients (i.e.,

small and less invasive sample collection) as well as increase sensitivity and accuracy. The clinical implications of biomarkers will continue to propel the healthcare field even further.

Conclusion

The use of biomarkers has revolutionized the healthcare industry, serving as indicators of various diseases, enabling early detection, accurate diagnosis, and personalized treatment plans [50]. The level of specificity and sensitivity required within each biomarker detection method ensures precise and rapid results. As discussed above, the types of biomarkers include molecular biomarkers, such as proteins and DNA; physiological biomarkers, like blood pressure; histological biomarkers observed in tissue samples; and radiographic biomarkers that utilize imaging techniques, including MRI and CT scans. Significant biomarkers that marked pivotal turning points were the discovery of HER2 in breast and pancreatic cancer, BCR-ABL in chronic myeloid leukemia CML, and LDL levels in cardiovascular diseases such as atherosclerosis. Although this literature review focused on a small set of detection methods, many emerging technologies and processes are currently under development specifically for detecting these biomarkers. For example, companies are currently trying to detect cancer and other diseases using just a blood test known as liquid biopsies that detect cancerous tumors [51].

However, despite their transformation in the industry, biomarkers still have many limitations. For example, some diagnostic biomarkers cannot distinguish between specific subtypes, and certain surrogate endpoints may not accurately represent long-term clinical outcomes.

Biomarker Use	Pros	Cons
Diagnosis	Enables early and often non-invasive detection of disease	May lack specificity; some markers require multiple conditions
Prognosis	Helps determine the patient's risk and likely outcomes	Prognostic value may vary across populations and disease stages
Surrogate Endpoint	Speeds up clinical trials, reducing cost and time	May not perfectly correlate with clinical outcomes

Table 2. Biomarker Usage: Pros and Cons: The primary uses of biomarkers include diagnosis, prognosis, and surrogate endpoints. The inherent advantages and limitations should be carefully considered when using these to inform clinical decisions [52] [53] [30].

The future of biomarkers is likely to continue to evolve, continuing with discovery, characterization, and detection with artificial intelligence (AI). AI can enable more sophisticated predictive models for disease progression and treatment responses based on complex biomarker profiles [54].

As new therapeutic technologies are developed for novel diseases with no current treatment, new biomarkers will need to be continuously discovered to inform these therapies efficiently. Along with the discovery of new and more useful biomarkers, detection methods will also continue to advance, providing sensitive and accurate measurements. Similarly, as genetic sequencing becomes more affordable and the expansion of genomic databases continues to develop, incorporating genetic biomarkers into disease prediction, diagnosis, and prognosis will need to be better understood. The rapid advancement of biomarkers in a relatively short period has increased the likelihood of biomarkers becoming even more prevalent in the future of disease diagnosis and treatment. As new biomarkers are discovered and detection technologies continue to improve, their expanding use in diagnosing and identifying diseases will likely pave the way for enhancing patient care.

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