**Challenges and Opportunities of Biomarkers for Biological Targeting: A Potential Way of Diagnosis and Treatment**

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**ABSTRACT**

Biomarkers provide new avenues for studying disease processes and how drugs operate to combat disease. This information may be applied in the practice of evidence-based medicine to enhance illness diagnosis, the safety and efficacy of existing drugs, and the development of novel medicines and targeted treatments. Novel molecular biomarkers have the potential to change much of the present healthcare model, changing the emphasis from a reactive 'one-size-fits-all' approach to one that is more proactive and precise. In this new, proactive approach, disease or disease susceptibility may be diagnosed earlier, and disease may be controlled or possibly prevented before it begins; and when the disease is detected, new biomarker-based diagnostics may be used to develop treatment strategies that are tailored to the individual. Biomarkers may increase patient well-being in the long run by delivering improved health outcomes. Health-care expenses are growing in OECD nations and across the world, and are expected to rise further as the population grows, people live longer, and the frequency of chronic and infectious illnesses rises. Novel biomarkers, whose discovery and development have been hastened by a decade of investment in genomics research, have the potential to improve health outcomes and lower total health care expenditures in the long run.

**Keywords-** Biomarkers; disease; diagnosis; surrogate endpoints; clinical endpoints; drug development.

**I. INTRODUCTION**

Biomarkers are objectively quantifiable markers of biological conditions. Biomarkers in health care can increase our understanding of disease and give information on disease existence or susceptibility in an individual, as well as predict or monitor patient response to treatment interventions. The application of innovative molecular biomarkers in the practise of evidence-based medicine may enhance illness diagnosis or therapy, increase health outcomes, and lowering disease's social and economic cost [1].

According to the National Institutes of Health in the United States, a biomarker is "a trait that is objectively measured and assessed as an indication of normal biological processes, pathogenic processes, or pharmacologic reactions to a therapeutic intervention." Biomarkers can include cellular properties, metabolites (such as sugars, lipids, and hormones), molecular changes, or physical traits (such as clinical symptoms) and are evaluated via measurement, annotation, documentation, and photographs. The identification of new biomarkers is becoming more linked to breakthroughs in molecular biology methods that may be accessible through DNA, RNA, or protein analysis [1].

We can discriminate four main types of molecular biomarkers:

• Genomic biomarkers: based on the analysis of DNA (deoxyribonucleic acid) profiles, especially the analysis of SNPs (single nucleotide polymorphisms), i.e. identification of punctual variations in genomic DNA.

• Transcriptomic biomarkers: based on the analysis of RNA expression profiles.

• Proteomic biomarkers: based on the analysis of the protein profiles.

• Metabolomic biomarkers: based on the analysis of metabolites (metabolites are the intermediates and products of metabolism) [2].

The Biomarkers Definitions Working Group of the National Institutes of Health defined a biomarker as "a trait that is objectively measured and analysed as an indication of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" in 1998 [2].

Biomarker discovery is accelerating at an unprecedented rate, thanks to recent investments in genetic science that are allowing for a better understanding of disease causes and unique patient responses to therapy. Such biomarkers enable earlier illness detection, improved diagnoses, and safer and more efficacious treatments, resulting in better patient outcomes and more efficient and effective public health spending. Promising findings from early applications of biomarkers show that, given the appropriate conditions, their incorporation into evidence-based medicine has the potential to revolutionise our approach to chronic illness and other major disorders, altering how disease is identified and treated. Securing the necessary circumstances for biomarker acceptance across health systems remains difficult, but doable. Those countries that successfully integrate biomarkers into their health-care systems stand to gain significantly [3].

**II. TYPES OF BIOMARKERS**

**Table 1:** Biomarkers categories

|  |  |  |
| --- | --- | --- |
| Biomarkers category | Description | Examples |
| Diagnostic | Presence of disease or condidtion or intify individual subtype of disease detect or confirm by biomarkers | For confirming cystic fibrosid swaet chloride is used as a biomarkers |
| Monitoring | A biomarker that is assessed repeatedly to determine the state of a disease or medical condition or to demonstrate susceptibility to (or the impact of) a therapeutic agent or an external factors. | The threshold of monoclonal protein (M protein) in blood may be used as a surveillance biomarker to ascertain whether people with monoclonal gammopathy of interstitial cells (MGUS) are displaying symptoms of progressing to other disorders, such as some blood types cancer, which may necessitate therapies. |
| Pharmacodynamic/ response | A biomarker that indicates the presence of a biological reaction in a person who has been exposed to a medicinal product or an environmental contaminant. | When examining individuals with hypercholesterolemia, serum LDL cholesterol can be utilised as a pharmacodynamic/response biomarker to measure response to a lipid-lowering drug or dietary modifications. |
| Predictive | A biomarker that promotes individuals who are more likely than likeminded individuals who do not have the biomarker to have a positively or negatively reaction to a therapeutic agent or a complex molecule. | When screening women with platinum-sensitive ovarian cancer, BRCA1/2 mutations may be utilised as predictive biomarkers to identify individuals who are likely to react to poly (ADP-ribose) polymerase (PARP) antagonists. |
| Prognostic | A biomarker also used predict the occurrence of a clinical event, illness repetition, or development in patients with the medical condition or disease of interest. | When examining women with breast cancer, BRCA1/2 mutations may be utilised as prospective indicators to predict the likelihood of another breast cancer. |
| Safety | A biomarker that is tested prior to or after exposure to a pharmaceutical preparation or a complex molecule to recognize the presence, probability, or amount of cytotoxicity as an undesirable impact. | When screening patients on medicines that influence kidney function to monitor for nephrotoxicity, serum creatinine may be utilised as a safety biomarker. |
| Susceptibility/risk | A biomarker that signals the possibility of acquiring an illness or health condition in a person who does not already have the disease or medical issue. | Variations in the Apolipoprotein E (APOE) gene may be utilised as biological predisposition biomarkers to locate individuals who are vulnerable to Alzheimer's disease. |

A biomarkers is not to study about a person feeling, living cycle, or functioning, a clinical outcome assessment measure (COA). The food and drug administration – national institues of health Biomarker working group created the BEST Resource in 2016 with the goal of clarifying and harmonising language and thereby speeding up research, development, and testing on innovative techniques, particularly biomarkers. Each biomarker, according to the BEST Resource Dictionary, falls into one of seven distinct groups [4].

Timeline

Description automatically generated with low confidence**Fig 1.** Drug exploration and research procedures with biomarker capability

**III. PATHWAYS TO DISEASE AND THE Future IMPACT OF BIOMARKERS**

**Biomarkers types**: -Biomarkers of exposure: used in risk prediction

-Biomarkers of illness: used in diagnosis, monitoring and screening

Biomarkers are frequently used in risk prediction, screening, and diagnostic processes, and they offer distinct and obvious benefits. Many neurological illnesses are classified using either established clinical criteria or histology diagnosis. Biomarkers offer the ability at an early stage to diagnose neurological disease, to give a mechanism for homogenous disease categorization, and to further our understanding of disease causation. Biomarkers are frequently used in risk prediction, screening, and diagnostic processes, and they offer distinct and obvious benefits. [5].

**Pathway of Disease**

**Risk aspects Diagnosis and screening Prognosis**

***Orientation Potential Infection***

**Etiologic Exposure**

**Pathogenesis Biomarkers Disease**

**Fig**. Disease pathway and potential impact biomarkers

A valid biomarker should be:

* a main product of oxidative alteration that may be directly involved in disease progression;
* a stable product that is not subject to artefact induction or destruction during maintenance;
* representational of the equilibrium between oxidative damage creation and clearance (i.e. the steady state, but also potentially relevant to accumulated oxidative damage monitoring);
* determined by a precise, sensitive, reproducible, and robust method
* devoid of confounding dietary intake variables;
* obtainable in a target tissue or a suitable surrogate tissue, such as a leucocyte; and
* quantifiable within the detection limits of a validated analytical method [5].

**IV. BIOMARKERS VERSUS CLINICAL ENDPOINT**

By definition, biomarkers are objective, measurable aspects of physiological operations. They could be or may not correspond to a patient's perception and sense of well-life, and it's possible conceive quantifiable biological traits that don't correspond to patients' clinical states, or whose fluctuations are unnoticeable and have no influence on healthiness. It's also easy to conceive observable natal traits that vary so much among populations that they're almost useless as dependable indicators of illness or its absence. Clinical endpoints, on the other hand, are characteristics that indicate or describe how a research or clinical trial participant "feels, functions, or lives." In other words, they are characteristics that represent the health and well-being of a study subject from the subject's perspective. The goal of clinical practise is to reduce morbidity and death, not to change quantitative aspects of individuals' underlying biochemistry with no clear clinical implications. Similarly, people seek treatment for their illnesses, not for numerical indications that frequently but not always accurately correspond with them. Although many recognise continued existence to be the gold-standard clinical endpoint for most HIV trials, several well, unequivocal clinical factors, such as brain haemorrhage, infarction, and the incidence of predetermined secondary infections, are also considered as endpoints in proper situations; they offer straightforward, unequivocal data that can show conclusively whether interventions are effective or ineffective, as well as safe or unsafe. However, not all clinical endpoints are created equal; here are some instances of medical data items that provide less reliable outcomes. [6].

**V. BIOMARKERS AS SURROGATE ENDPOINT**

When used as clinical trial outcomes, biomarkers are referred to as surrogate endpoints; that is, they act as surrogates or substitutes for clinically important endpoints. Unfortunately, not all biomarkers are meant to be used as surrogate endpoints. Surrogate endpoints are a small subset of well-characterized biomarkers with therapeutic applications. To be called a surrogate endpoint, there must be persuading scientific evidence (e.g., epidemiological, pharmacological, and/or pathophysiological) that a biomarker reliably anticipates a clinical outcome, either benefit or harm. A surrogate endpoint is a biomarker that may be used to compensate for, but not replace, a clinical endpoint. [7].

**VI. CHARACTERIZATION AND EVALUATION OF BIOMARKERS**

When used as clinical trial outcomes, biomarkers are referred to as surrogate endpoints; that is, they function as surrogates or substitutes for clinically important endpoints. Not all biomarkers, however, are designed to be surrogate endpoints. Surrogate endpoints are a small group of well-studied biomarkers with medicinal value. To qualify as a surrogate endpoint, there must be strong scientific evidence (e.g., epidemiologic, pharmacology, and/or pathophysiology) that a biomarker easily and effectively predicts a clinical outcome, either advantage or harm. A surrogate endpoint is a biomarker that may be used to supplement but not replace a clinical objective [8].

Choosing biomarkers as surrogate endpoints demands determining their applicability and reliability. Relevance refers to a biomarker's ability to provide important clinical advice on topics of available to the public community, health providers, or health regulators. Validity refers to the requirement to characterise a biomarker's effectiveness or usability as a surrogate endpoint. Regrettably, authenticity is rarely binary, but rather a spectrum. Indeed, some academics have deemed the term "confirmation" "inappropriate" for the research of biomarkers since it presupposes a detailed scientific overview of the relationship among a given biomarker and a clinical result, which they find unacceptable [9].

For many years, researchers used atrial fibrillation inhibition as a surrogate endpoint for reduced comorbidities from coronary heart disease, actually results in the authorization of anti-arrhythmia drugs (e.g., encainide, flecainide, moricinze) that were later proven to raise death rates in some clinical situations in subsequent trials. More awhile back, a large and is well trial of the mixture of two cholesterol-lowering drugs, docetaxel and lovastatin, demonstrated the dangers of excessive reliance on biomarkers: whereas the combination therapy reduced participants' bad cholesterol more often than simvastatin on its own, it did not reduce endothelial dysfunction or mortality risk, putting into question much prior findings that depended on the presumption that taking supplements would enhance atherosclerosis or mortality risk [9].

**VII. OCCURRENCE BIOMARKERS OR PREDICTOR BIOMARKERS**

Environmental exposures, effect modifiers, or risk factors When a hazardous exposure is anticipated of causing disease, researchers obviously want to understand how much exposed occurred. External exposure refers to the amount of the poison in a person's surroundings. While questionnaires include a continuous account of publicity, quantity of the suspicion toxicant in the air, water, soil, or food might provide accurate data on the "dosing regimen" of publicity. The outside dose measurement provides the framework for comprehending the relationship to the sickness process, however the "internal" dose measurement may provide more precision. Toxins detected in organs or physiological liquids serve as biomarkers for pre - validated. A biomarker that identifies a "biochemically optimal dose" indicates the amount of poison or substance found in the tumor site or surrogate. Exposure to lead is an excellent example. Lead in the environment can confirm a background of exposure to lead, but blood and organs offer the most accurate indicator of the dose of publicity (hair, nails, teeth). Although different body fluids may be used depending on the pharmacologic properties of the drug, the hemodynamic properties of the toxin or chemical of concern must be considered when calculating the external dose. Some compounds, such as halogenated hydrocarbons, are kept in adipose tissue, while others, such as organophosphorus insecticides, must be evaluated in blood or urine. [10].

A biomarker of event, as opposed to a heritage of exposure, indicates the actual "internal" dose of the exposure. This improves precision in risk factor assessment by accounting for both validity and reliability when examining the impact of experience on fate. Biomarkers are especially helpful in the cross-sectional diagnosis of acute sickness due to the pharmacologic properties of the chemical or toxin. Finding biomarkers for persistent exposures across the long time spans required for randomized trials of chronic neurological illnesses such as Alzheimer's disease is particularly difficult. Banked serum or plasma may be beneficial in some situations, based on the condition being examined and the pharmacologic qualities of the biomarker [10].

**Genetic susceptibility**

Epidemiologic study may explore familial aggregation and assess genetic and environmental components to an illness using life table approaches and recurrence risk. Mendelian disease gene mutations are frequently foreseeable. Although not deterministic, variant alleles or polymorphisms in genes may be linked to susceptibility. The majority of adult-onset degenerative neural system diseases are assumed to be caused by a mix of inherited and environmental causes. The linked combinations of these qualities comprise the characteristic or sickness. As a result, these precursor biomarkers may or may not be involved in pathogenesis. In certain circumstances, genetic variation is not necessary nor sufficient to cause illness. They can, however, be effective precursors at any stage of the disease's progression [11].

**Intermediate biomarkers**

Some biomarkers tangible outcomes steps in the illness's causal chain and are thus intimately associated to sickness. Others are only tenuously linked to the cause. There are various alternatives to consider. A biomarker may be reliant on another known or unfamiliar ingredient to cause illness. As a consequence, although it is not the only predictor, it is a factor of the causality route and remains tightly correlated with the condition. The biomarker might be linked to a prior knowledge exposure or indicate a modification caused by the exposure that resulted in the illness. The most perilous situation is when the biomarker is linked with a whenever one that is also associated with the exposed [12].

**VIII. BIOMARKERS' ROLE IN DIAGNOSIS**

Tests for prognosis, screening, and diagnosis Prodromal biomarkers enable for earlier detection or identification of the outcome of interest at a more basic stage of disease. Blood, urine, and cerebrospinal fluid offer the biological information needed for the diagnosis. In certain cases, biomarkers are employed to identify a biological feature that suggests whether it’s a subclinical symptom, stage of the ailment, or a surrogate manifestation of the disease. Biomarkers used for screening or diagnosis are frequently used to represent surrogate indicators of sickness.The potential uses of this class of biomarkers include:

1) Individuals who are doomed to be affected or who are in the "preclinical" phases of the sickness are identified.

2) Reduction of illness heterogeneity in clinical trials or epidemiologic investigations,

3) Reflection of disease biological sciences, including initiation, concealment, and identification, and

4) Plan a clinical experiment. The gains in validity and accuracy offset the difficulties in acquiring such tissues from individuals. [13].

Most ethical review boards and healthcare systems require thorough obey for individuals who screened positive, irrespective of whether they have the condition. People who test positive should be provided therapy that is both affordable and appropriate. Those who carry genes and are unwell should be given a choice of appropriate and readily available medicines. It is vital to highlight that the main benefit of screening is avoidance, either primary (before problems appear) or tertiary (after symptoms arise) (early or prodromal identification). Consider the benefits of performing a therapeutic trial in patients before they exhibit overt symptoms. Neurological diagnostic tests are increasingly being used in clinical research and practise. The gathering of information from many sources, some of which include diagnostic test findings, assists in the ultimate goal of increasing the likelihood of a specific diagnosis. Clinical tests are also performed, but probably less frequently, for other purposes such as determining the severity of an illness, anticipating disease development, or evaluating response to a certain drug. More importantly, disease biomarkers lend themselves easily to clinical trials. Additional benefit of this type of diagnostic test is that it minimises disease heterogeneity in clinical trials or observational epidemiologic research, resulting in a better understanding of the natural history of sickness [13].

**Variability**

Although there are several benefits to employing biomarkers, inconsistency is a major issue. Whether the biomarker is an exposure or effect modifier, a disease surrogate, or an indication of susceptibility, variability occurs. The amount of an external exposure or how a suspected toxin is metabolised might produce interindividual differences. Individuals exposed to the same chemical may differ in their ability (or inability) to metabolise the substance, or they may have had different types of exposures (in the field as compared with in the office). Intraindividual variability is often connected with laboratory errors or other individual-specific characteristics or exposures. Group elsewhere throughout as well, but this is often the intended outcome of a research. Naturally, it works best when the disparities between groups are considerable. Regardless, specificity and sensitivity or comparable variance estimates, are used to evaluate a biomarker's ability to differentiate between groups. The likelihood of exposure misrepresentation is minimized when the sources of variability in biomarker measurement are considered. While measurement error with biomarkers is constantly a challenge, other variables may explain individual or group variation [14].

**Validity**

Exact figures are alluring, but they pose the same problems as any variable. The accuracy, relevance, tolerance, specificity, imputation bias, and interpretation of marker data should be examined in the same manner that any other variable is. These difficulties continue whether the biomarker is used as a variable in a clinical trial or an epidemiological study. The importance of dependability and predictability cannot be overstated [15].

**Biomarkers for IEM (Inborn errors of metabolism)**

* A biomarker is a detectable disease trait or analyte that may be used to accurately assess the existence, degree, and clinical progression of a disease. An ideal biomarker assesses disease activity indirectly and may aid in therapeutic care. A biomarker should be easily detectable in clinical samples. In the general population, the levels should not differ considerably. Biomarker levels should also be related to disease progression and treatment response. The desired biomarkers should also be easily and quickly measured. Biomarkers used in screening, diagnosis, and therapy monitoring

Some Biomarkers Used in IEM Disease Biomarker(s)

* **Gaucher disease**: Chitotriosidase pulmonary and activation-regulated chemokine (PARC/CCL18)Macrophage inflammatory proteins (MIP-10and MIP-10)
* **Fabry disease**; Globotriaosylsphingosine (lysoGb3) Globotriaosylceramide (Gb3)
* **Mucopolysaccharidoses:** GAGsHeparin cofactor II thrombin (HCII-T) Serum dipeptidyl peptidase-IV (DPP-IV) [16].

**Biological biomarkers**

Biomarkers of exposure are classified into two types: internal dose and physiologically effective dose. Internal dosage biomarkers seek to identify the substance or its metabolites in tissues or bodily fluids such as blood, urine, breast milk, and saliva [17].

They can also provide information on alternative routes of exposure to that drug as well as the existence of metabolic enzyme genetic polymorphisms. Biomarkers of physiologically effective dosage evaluate substances' interactions with drug target such as DNA and protein receptors (e.g., measurement of DNA and protein adducts in urine and serum). Regardless of the ease with which these adducts may be quantified, DNA dimers have grown in popularity as one of the most significant biomarkers of exposure since their existence may indicate the risk related to the exposure. Although biomarkers of risk are very relevant and specific signals of an exposure, the information provided does not always convert into a prediction of medical complications, hence alternative biomarkers must be investigated [17].

**Renal biomarkers**

In recent years, the number of renal BMs has significantly risen, ranging from BMs or renal function such as blood urea nitrogen and creatinine to Bristol Myers Squibb (BMS) of tubular health. These BMs can be measured in the urine or blood. Urinary samples are routinely collected overnight from animals in metabolism cages on Nalgene micro chiller blocks and frozen at 80°C. -glutathione S transferase (aGST), kidney injury molecule 1 (KIM1), lipocalin-2 (Lcn2; also known as neutrophil gelatinase associated lipocalin), micro-albumin, osteopontin (Spp1), clusterin (Clu), trefoil factor 3 (TFF3), and renal papillary antigen are all often measured in urine. Electro chemiluminescent tests can detect them [18].

**Table 2**: Biomarkers of urine for renal injury and disease

|  |  |  |
| --- | --- | --- |
| *Biomarkers* | *Performance* | *Lesions monitored* |
| Albumin, K1M1, Clu | Many exceed and provide information to blood urea nitrogen (BUN) and SCr. | Chronic kidney capillary changes in rats |
| B2M, Cystatin C, and total protein | Individually, it may outperform the SCr assay and provide information to the BUN and SCr assays. | Early indications of acute drug-induced glomerular changes or injury in rats, leading in impaired kidney tubular digestion and absorption |
| TFF3 | BUN and SCr tests in rats are enhanced. | Early signs of acute drug-induced glomerular alterations or damage in rats, which resulted in decreased kidney tubular digestion and absorption |

**Hepatic Biomarkers**

The liver function may be assessed utilising panels of BM found in the serum. The use of ROC analysis to screen for certain forms of liver histopathology resulted in the discovery of parameters thought to be meaningful indicators of hepatic damage. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (Tbili), and serum bile acids (SBA) have the most predictive usefulness for hepatocytes necrosis and biliary damage, with equal magnitudes of area under the ROC curve and serum hepatobiliary biomarker alterations. ALT elevations with biochemical or morphological signs of cholestasis were reported in the context of hepatocellular necrosis. After being exposed to hepatotoxicants (CHCl3 and CCl4), the activity of AST and ALT, which are markers of liver injury, increased dramatically [19].

**Cardiac Biomarkers**

Major advances in the field of cardiac BMs have been achieved in recent years, with the focus focusing on recognising BMs of myocardial necrosis. This fast advancement is due in part to the introduction of supersensitive tests for cardiac troponins I (cTnI) and T. (cTnT). Cardiac proteins found are mechanical apparatus proteins that are particular to the heart, and elevations in their blood level have long been associated with myocardial necrosis [20].

**Skeletal Muscle**

Biomarkers Muscle toxicity can be caused by a number of medicines, including statins, PPAR-agonists, steroids, and -adrenergic receptor agonists. Such substance skeletal muscle poisoning can result in considerable liability, as demonstrated by the recall of cerivastatin (Baycol), a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, from the market after it was linked to 100 rhabdomyolysis-related fatalities. Nonclinical identification of skeletal muscle toxicity generally relies on conventional histopathological examination, which is supported by serological BMs like as creatine kinase (CK) and AST [21].

**IX. Advantages and disadvantages of biomarkers**

**Implementation difficulties**

The use and spread of biomarkers within a brand-new healthcare paradigm could be transformational and alter how diseases are recognised and treated. However, there are a number of obstacles that must be overcome beforehand personalized medication may become authenticity in the translation of biomarkers into clinical practice [22]:

1. The discovery of clinically useful molecular biomarkers. Data is being generated by genomic and related technologies and instruments more quickly than it can be analyzed and interpreted for use in a scientific framework. In fact, the hunt for original molecular biomarkers is a very expensive and complex endeavour that frequently exceeds the capabilities of any one actor. Progress is nevertheless gradual and not as effective or efficient as it could be, despite the construction and organisation of massive infrastructures set up to gather and share knowledge to stimulate biomarker discovery and validation.

2. Demonstrating the clinical value and validity of tests based on biomarkers. Evidence of the biomarker's clinical validity and utility will be needed before biomarker-based testing can be used in the clinical context. This is critical to ensure regulatory approval and adequate remuneration for the test, as well as to assist physicians in making decisions within the context of evidence-based medicine. But gathering the information required to demonstrate clinical validity and utility can be a time-consuming and expensive procedure that is made more challenging by the absence of standards.

3. Ineffective regulatory and payment frameworks. The regulatory and reimbursement processes in the majority of OECD nations are not well suited to deal with this new wave of molecular biomarker-based diagnostic tests. Modern biomarker-based diagnostics that produce complex data needing in-depth analysis and interpretation are not well adapted to the regulatory systems now in place because they were designed around simple diagnostic tests. Furthermore, present reimbursement procedures may not accurately account for the testing's complexity or its importance to the healthcare system. The adoption of these technologies in the clinical arena may be hampered by inadequately designed regulatory and reimbursement systems, which may also serve as a deterrent to further biomarker research.

4. Modifying the clinic's use of evidence-based medicine. The medical community may be reluctant to adopt or make use of novel clinical testing. The test's information must be understood by doctors so they can know how to use it in their decision-making. Patients must understand how new tests can help them and the ramifications of the new knowledge they may reveal. Like other genetic tests, biomarker-based assays have a variety of privacy concerns that must be resolved. The ease with which tests can be incorporated into doctors' current practises will also be reflected in their uptake in the clinical setting.

5. The absence of suitable business models. For innovations to proceed into the market arena successfully, there must be workable business models. The same is true for the introduction of innovations based on biomarkers in the medical field [22].

Depending on how you look at it, novel molecular biomarkers may be able to revitalise the diagnostic sector, which has been pretty stagnant. However, it is urgent to find workable business plans to promote the creation and commercialization of diagnostic tests based on biomarkers.

By enabling a more precise description of the target group that has the highest potential for a benefit and the lowest risk to experience unintended side responses, biomarkers aid in the development of medicines. This reduces healthcare spending and offers justification for reimbursement contracts. Overall, the proper use of biomarkers has the potential to dramatically speed up the approval process, minimise development costs, and improve the quality and safety of a medicine.

The development of biomarkers, however, is fraught with difficulties, including the following:

* The scientific basis for some biomarkers cannot always be verified, creating difficulties for biomarker certification and validation in the future. Additionally, it is important to minimise misinterpretations of biomarker data and incorrect associations between a biomarker and a disease.
* Development costs for biomarkers could rise as a result of more extensive clinical trials or extra testing requirements. Additionally, it is common for commercial drug companies to be reluctant to spend upfront in biomarkers and to release biomarkers data into the open market.
* The development and certification of biomarkers is often labor- and resource-intensive.
* For qualification purposes, more convincing evidence of a favourable benefit-risk analysis is typically needed than for an assessment as part of a regulatory clearance for a single medicine. This explains why academic groups and consortia, as opposed to lone commercial drug producers, more frequently pursue biomarker certifications from the EMA and FDA.
* For the development of "personalised pharmaceuticals," early population-related strategy decisions are necessary to ensure that a smaller group of eligible patients will still be able to earn a profit.
* Collaborations between industry, private organisations, academic institutions, and regulatory bodies are what drive the ongoing growth of the biomarker scientific and regulatory landscape. The acceptance of biomarkers has recently increased in the US and EU as a result of these cooperation. The acceptance of biomarkers is anticipated to increase further in the near future [24].

**Using biomarkers to their full potential**

This type of biomarker-based technology integration into the healthcare system has a number of potential advantages:

* Improved diagnostics may result in early disease identification and treatment, thereby enhancing health outcomes, and lowering both the direct and indirect costs of illness and care.
* Through the use of pharmacogenetics, medications may be made more safe and effective while also reducing unpleasant side effects.
* As medication development costs and deadlines are shortened by the use of biomarkers in the field of pharmacogenetics, more safe and effective medicines may become available. These modifications may also have favourable economic effects in addition to many advantageous results for patients and healthcare systems.
* Less variety in patient response and fewer side effects will lead to savings in the health care system as a whole; regulators and third-party payers may be at lower risk of adopting expensive pharmaceuticals.
* By using biomarkers to expedite drug delivery and assess the safety and effectiveness of those drugs, therapeutics developers may lower their financial risks and increase production. Despite the evident promise of biomarkers, there are still major obstacles to overcome [23].

For the discovery, development, and approval of novel drugs, biomarkers are essential instruments. Over 20% of the medications FDA authorised between 2014 and 2018 and about 42% alone in 2018 fall within the category of "personalised medicines." 68,78. Between 2015 and 2019, over 65% of therapeutic approvals by the EMA and FDA were linked to the inclusion of at least one biomarker in the development programme, and in the near future, a higher acceptance rate of biomarkers is anticipated. It is obvious that biomarkers are now a crucial component of medication development, even though this percentage depends on a number of variables, including current scientific advancement, characteristics of a product class, and development of the regulatory landscape. Several advantages for patients:

* Biomarkers are widely utilised in diagnostics, medication research, and development, and can be helpful at every stage of the process, from creating an animal model that is acceptable to choosing patients who will be suitable for clinical trials and differentiating them from rivals.
* Biomarkers assist in choosing the best therapeutic candidates, which considerably lowers the cost of discovery and the likelihood of failure in subsequent stages.
* Biomarkers can aid in a better understanding of the mechanism of action, allowing for the prediction of unfavourable side effects and drug-drug interactions (DDIs).
* Because fewer participants are required to demonstrate clinical benefit and non-inferiority, biomarkers offer the potential to minimise the number of patients in clinical trials. Using the right biomarkers to stratify patients can lower the risk of failure due to safety and effectiveness problems.
* Biomarkers might be employed as a clinical study's stand-in endpoint. 27% of medications that the FDA authorised between March and May 2016 employed at least one surrogate measure as a main endpoint. For instance, the approval of the HIV-1 infection therapy drug Odefsey was predicated on the viral suppression and CD4+ cell counts as surrogate markers.
* Biomarkers make it easier for regulatory bodies to decide how to proceed with a drug development project based on the medicine's benefit-risk profile [23].

**X. Current scenario for the diagnosis**

Potential biomarkers are being investigated from a broad and expanding range of biological measurements, including many domains and degrees of analysis. These tests include omics analyses of blood, cerebrospinal fluid (CSF), and other tissues, electrophysiology in peripheral nerves and the brain, and structural and functional imaging of the brain and peripheral tissues. In clinical trials and clinical treatment for pain, there are currently few biomarkers based on electrophysiology, omics, and imaging, while significant validations are starting to emerge [24].

**Electrophysiological biomarkers**

Electrophysiology can show electrical signals connected to pain that are sent from peripheral nerves to the brain. Microneurography61–63, as well as tests on neurons or non-neural cells produced from induced pluripotent stem cells, are examples of peripheral measurements. EEG and related magnetoencephalography measurements of evoked potentials and oscillations in pain-related brain systems are among the brain measurements [26].

**A test called Oncotype® DX**

In 2004, Genomic Health released Oncotype DX to the public. A panel of 21 genes within a tumour are simultaneously examined by the validated genomic test Oncotype DX. These genes serve as breast cancer biomarkers. The chance of disease recurrence in women with early-stage breast cancer may be quantified using statistical analysis of the level of expression of these 21 genes, and the likelihood that particular types of chemotherapy will be beneficial can also be determined. The first gene expression test recognised for its ability to predict a patient's response to chemotherapy and recurrence risk is called Oncotype DX. Oncotype DX is based on several research by Genomic Health that demonstrate. This has made it much easier for regulatory bodies to approve it. Numerous studies including more than 3 300 patients examined Oncotype DX, and a cost-effectiveness study was also carried out. Genomic Health subsequently provided information on the test's reliability, clinical value, and financial efficiency when used in clinical practise. The clinical and economic assessments were both favourable. Many health insurance providers in the United States are now paying for this complicated test, even at its current list price of USD, as a result of this kind of examination [27].

**Omic-based biomarkers**

Because they provide biological markers from easily accessible body compartments, omic techniques are appealing. Because blood analysis is common, minimally intrusive, and reasonably priced81, metabolites, proteins, or DNA detected in blood are the primary biomarkers employed in clinical practise today. Clinically available omic biomarkers for pain treatment have the potential to improve patient care and reveal pathophysiological causes. Such a prospect, meanwhile, is now mostly supported by exploratory data and would need to be carefully examined in sizable, diverse, and well-phenotyped patient populations.Whether omic signatures derived from readily available biospecimens, such as blood, urine, CSF, or exudate, reflect pertinent biology is a crucial question, as is determining which pain conditions — such as inflammatory versus neuropathic, or temporal patterns from acute to chronic — would benefit from such profiling [28].

**Herceptin/HER2 screening and Herceptin in breast cancer: pharmacogenetic testing**

Herceptin and HER2 were important early examples of breast cancer prognostic biomarkers and show the possibility of individualised treatment. Women with metastatic breast cancer whose tumours overexpress the protein HER2 are treated with Herceptin®. Due to each person's unique genetic makeup, one in every four breast cancer patients has HER2 positivity. It is feasible to recognise and treat those who will benefit from Herceptin® by testing all women diagnosed with breast cancer for the hereditary biomarker HER2.The effectiveness of Herceptin® has been seen to enhance with this focused use. As a result, the percentage of women in the targeted group who will respond to the medication is significantly higher than the percentage of women with breast cancer overall who would benefit from it. The risk-benefit ratio of the treatment can be altered by this strategy since fewer patients will have adverse effects (or no therapeutic effect) because the medication is only given to women who are likely to benefit from it. Herceptin®, which had sales of USD 747 million in 2005, is the first example of a successful personalised drug. About 37 customised medications and the tests that go along with them (also known as pharmacogenetic tests) have been available since 2005 [29].

**Role of biomarkers for a new health care paradigm**

An ageing population and an increase in the use of pricey technologies like magnetic resonance imaging (MRI) and computed tomography (CT) scans are two major factors contributing to an increase in health care costs across the OECD countries, which have grown at a rate of 4% annually over the last decade and now amount to almost 9% of GDP. Despite these costs, the OECD's health system still requires reform. More than 12% of hospitalised patients in Norway experience adverse events, 70% of which could have been avoided, and more than half of which result in disability; in England, it is thought that more effective primary care could have prevented more than 40%, or nearly 1.7 million emergency admissions to hospitals. 5 cost of medical treatment in the Governments all around the world are struggling to strike a balance between their citizens' demands of the greatest healthcare and the necessity for cost control and growth in the developing countries [29].

**The pharmaceutical context**

In all OECD nations, pharmaceuticals play a crucial role in the prevention and treatment of disease and have significantly improved patient care. Pharmaceuticals are an effective way to cure many fatal and disabling diseases, and they have greatly enhanced life expectancy in the OECD countries. But during the past ten years, the procedure for finding new drugs and developing them has grown more time-consuming and expensive.. Withdrawals from well-known drugs like Vioxx have made the issue worse. From target selection to clinical application, medication development takes an average of 12 years and costs between USD 350 million and USD 1 billion. Poor target identification and validation as well as the failure (attrition) of compounds late in the research phase are major contributors to expense. Costs and attrition seem certain to rise as business is forced to concentrate its efforts on more challenging disease targets, particularly those linked to complex disorders like cancer, diabetes, and asthma [29].

**The clinical context**

Evidence-based medicine has already benefited significantly from the clinical application of biomarkers. The amount of diagnostic and pharmacogenetics-based tests that are currently offered to support physicians' decision-making process serves as the finest example of this. In order to diagnose latent or subclinical disease, these tests can

1. Help in diagnosis of latent or subclinical disease;
2. Assist in determining who will respond and not respond to a treatment;
3. Assist in determining the proper doses for responders; and
4. Determine the risk of harmful drug effects or adverse drug reactions, potentially eliminating some patients from treatment [30] .

**XI. Biomarkers: Uses and Restrictions**

The biomarker is insufficient. In order to demonstrate a reasonable level of reliability, pilot studies should be carried out. The dependability of the biomarkers employed in any inquiry may be impacted by modifications to laboratory personnel, methodology, storage, and transit practises. To evaluate test-retest agreement and consistency, employ kappa statistics for binary or dichotomous data and intraclass correlation coefficients. A biomarker's validity is complicated to assess. Three aspects of measurement validity are proposed by Schulte and Perera:

1) Content validity, which demonstrates how closely a biomarker corresponds to the biological process under investigation,

2) Construct validity, which relates to additional pertinent traits or aspects of the disease, such as additional biomarkers or disease signs,

3) Criterion validity, which is often assessed by sensitivity, specificity, and predictive power, demonstrates how much the biomarker connects with the particular disease [31].

Confidentiality, accuracy, and foreseeable future are all evaluated. In order to thoroughly check the effect of illness misdiagnosis, false positives and incorrectly classified, as well as positive and negative predictive power, should be examined. In an ideal scenario, the biomarker would be highly predictive, but that's not always the case. When combined with other testing, receiver-operator operator dynamic characteristics can provide the tools needed to select the best alternative in terms of sensitivity and untrue rates. It is critical to determine which choice will have the high specificity and lowest false-positive rate, especially when multiple tests are employed. Most individuals who are positive would agree that tests would indeed be great for chronic and progressive disorders. One purpose of screens is early identification with the goal of entirely treating the condition. Many of the techniques and difficulties that applicable to testing and treatment apply to screening as well. Detection rate, like some of the other medical testing, disclose details well about test's efficiency but not its chance of identifying a condition. To do so, we should evaluate the predictive validity (positive and negative) [31].

Diagnostic tests are meant to enhance medical assessment by raising the chance of illness, therefore the pretest probability would be high by design. Because the probability is considerably lower for screens, the PPV will be lesser. As a result, frequency, or the probability of disease in the past, should be seriously evaluated throughout screening. These analysis tools are now available in a variety of statistical packages. The investigator must disclose its use of the biomarker in the study. Over interpreting biomarker data is the main cause of errors. The most common source of mistakes is incorrect interpretation of biomarker data. For example, the outcomes of one study may imply that a certain biomarker (measured as a measure of exposure or susceptibility) is strongly connected to a specific illness or outcome. On the other side, the researcher interprets the discovery as a biomarker for the sickness or the observed outcome. A biomarker of this type cannot be expected to act as a diagnostic test until it manifests as a disease, regardless matter how high the odds ratio or relative risk is. For example, the APOE-4 allele is strongly associated to Alzheimer's disease, although its presence does not indicate the condition [31].

**XII. Reference**

1. Strimbu, K., & Tavel, J. A. (2010). What are biomarkers?. *Current Opinion in HIV and AIDS*, *5*(6), 463.
2. Griffiths, H. R., Møller, L., Bartosz, G., Bast, A., Bertoni-Freddari, C., Collins, A., & Astley, S. B. (2002). Biomarkers. *Molecular aspects of medicine*, *23*(1-3), 101-208.
3. Arbitrio, M., Scionti, F., Di Martino, M. T., Caracciolo, D., Pensabene, L., Tassone, P., & Tagliaferri, P. (2021). Pharmacogenomics biomarker discovery and validation for translation in clinical practice. *Clinical and Translational Science*, *14*(1), 113-119.
4. Gromova, M., Vaggelas, A., Dallmann, G., & Seimetz, D. (2020). Biomarkers: opportunities and challenges for drug development in the current regulatory landscape. *Biomarker insights*, *15*, 1177271920974652.
5. Aronson, J. K., & Ferner, R. E. (2017). Biomarkers—a general review. *Current protocols in pharmacology*, *76*(1), 9-23.
6. DeGruttola D, Fleming TR, Lin DY, Coombs R. (1997). Perspective: Validating surrogate markers: Are we being naïve? J Infect Dis, 127:237–246.
7. Aronson, J. K. (2005). Biomarkers and surrogate endpoints. *British journal of clinical pharmacology*, *59*(5), 491.
8. Pruett, S., Hébert, P., Lapointe, J. M., Reagan, W., Lawton, M., & Kawabata, T. T. (2007). Characterization of the action of drug-induced stress responses on the immune system: evaluation of biomarkers for drug-induced stress in rats. *Journal of immunotoxicology*, *4*(1), 25-38.
9. Bravo-Merodio, L., Acharjee, A., Russ, D., Bisht, V., Williams, J. A., Tsaprouni, L. G., & Gkoutos, G. V. (2021). Translational biomarkers in the era of precision medicine. *Advances in clinical chemistry*, *102*, 191-232.
10. Chen, C. J., Hsu, L. I., Wang, C. H., Shih, W. L., Hsu, Y. H., Tseng, M. P., ... & Wu, M. M. (2005). Biomarkers of exposure, effect, and susceptibility of arsenic-induced health hazards in Taiwan. *Toxicology and applied pharmacology*, *206*(2), 198-206.
11. Norppa, H. (2003). Genetic susceptibility, biomarker respones, and cancer. *Mutation Research/Reviews in Mutation Research*, *544*(2-3), 339-348.
12. Knudsen, L. E., & Hansen, Å. M. (2007). Biomarkers of intermediate endpoints in environmental and occupational health. *International journal of hygiene and environmental health*, *210*(3-4), 461-470.
13. Branca, F., Hanley, A. B., Pool-Zobel, B., & Verhagen, H. (2001). Biomarkers in disease and health. *British Journal of Nutrition*, *86*(S1), S55-S92.
14. Meijers, W. C., van der Velde, A. R., Muller Kobold, A. C., Dijck‐Brouwer, J., Wu, A. H., Jaffe, A., & de Boer, R. A. (2017). Variability of biomarkers in patients with chronic heart failure and healthy controls. *European journal of heart failure*, *19*(3), 357-365.
15. Dor, F., Dab, W., Empereur-Bissonnet, P., & Zmirou, D. (1999). Validity of biomarkers in environmental health studies: the case of PAHs and benzene. *Critical reviews in toxicology*, *29*(2), 129-168.
16. Mamas, M., Dunn, W. B., Neyses, L., & Goodacre, R. (2011). The role of metabolites and metabolomics in clinically applicable biomarkers of disease. *Archives of toxicology*, *85*(1), 5-17.
17. Hughes, M. F. (2006). Biomarkers of exposure: a case study with inorganic arsenic. *Environmental health perspectives*, *114*(11), 1790-1796.
18. Trof, R. J., Di Maggio, F., Leemreis, J., & Groeneveld, A. J. (2006). Biomarkers of acute renal injury and renal failure. *Shock*, *26*(3), 245-253.
19. Amacher, D. E. (2002). A toxicologist's guide to biomarkers of hepatic response. *Human & experimental toxicology*, *21*(5), 253-262.
20. Singh, V., Martinezclark, P., Pascual, M., Shaw, E. S., & O'Neill, W. W. (2010). Cardiac biomarkers–the old and the new: a review. *Coronary artery disease*, *21*(4), 244-256.
21. Aldous, S. J. (2013). Cardiac biomarkers in acute myocardial infarction. *International journal of cardiology*, *164*(3), 282-294.
22. Gromova, M., Vaggelas, A., Dallmann, G., & Seimetz, D. (2020). Biomarkers: opportunities and challenges for drug development in the current regulatory landscape. *Biomarker insights*, *15*, 1177271920974652.
23. Schuster, D. P. (2007). The opportunities and challenges of developing imaging biomarkers to study lung function and disease. *American journal of respiratory and critical care medicine*, *176*(3), 224-230.
24. Davis, K.D., Aghaeepour, N., Ahn, A.H. *et al.* (2020).Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: challenges and opportunities. *Nat Rev Neurol* ,*16* , 381–400
25. Deyati, A., Younesi, E., Hofmann-Apitius, M., & Novac, N. (2013). Challenges and opportunities for oncology biomarker discovery. *Drug discovery today*, *18*(13-14), 614-624.
26. Jeste, S. S., Frohlich, J., & Loo, S. K. (2015). Electrophysiological biomarkers of diagnosis and outcome in neurodevelopmental disorders. *Current opinion in neurology*, *28*(2), 110.
27. Duffy, M. J., Harbeck, N., Nap, M., Molina, R., Nicolini, A., Senkus, E., & Cardoso, F. (2017). Clinical use of biomarkers in breast cancer: Updated guidelines from the European Group on Tumor Markers (EGTM). *European journal of cancer*, *75*, 284-298.
28. Ng, S., Strunk, T., Jiang, P., Muk, T., Sangild, P. T., & Currie, A. (2018). Precision medicine for neonatal sepsis. *Frontiers in molecular biosciences*, *5*, 70.
29. Pedersen, H. C. B., & Bartlett, J. M. (2020). Predictive Markers for Targeted Breast Cancer Treatment. *Pharmacogenetics of Breast Cancer*, 135-149.
30. Sethi, S., Ali, S., Philip, P. A., & Sarkar, F. H. (2013). Clinical advances in molecular biomarkers for cancer diagnosis and therapy. *International journal of molecular sciences*, *14*(7), 14771-14784.
31. Mayeux, R. (2004). Biomarkers: potential uses and limitations. *NeuroRx*, *1*(2), 182-188.