**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 | A biomarker used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease | Sweat chloride may be used as a diagnostic biomarker to confirm cystic fibrosis8 |
| Monitoring | A biomarker measured serially for assessing status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent | Monoclonal protein (M protein) level in blood may be used as a monitoring biomarker to evaluate whether individuals diagnosed with monoclonal gammopathy of undetermined significance (MGUS) are showing signs of progressing to other disorders, including some types of blood cancer which may require treatment |
| Pharmacodynamic/ response | A biomarker used to show that a biological response has occurred in an individual who has been exposed to a medical product or an environmental agent | Serum LDL cholesterol may be used as a pharmacodynamic/response biomarker when evaluating patients with hypercholesterolemia, to assess response to a lipid-lowering agent or dietary changes |
| Predictive | A biomarker used to identify individuals who are more likely than similar individuals without the biomarker to experience a favourable or unfavourable effect from exposure to a medical product or an environmental agent | BReast CAncer genes 1 and 2 (BRCA1/2) mutations may be used as predictive biomarkers when evaluating women with platinum-sensitive ovarian cancer, to identify patients likely to respond to poly (ADP-ribose) polymerase (PARP) inhibitors |
| Prognostic | A biomarker used to identify likelihood of a clinical event, disease recurrence or progression in patients who have the disease or medical condition of interest | BReast CAncer genes 1 and 2 (BRCA1/2) mutations may be used as prognostic biomarkers when evaluating women with breast cancer, to assess the likelihood of a second breast cancer |
| Safety | A biomarker measured before or after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect | Serum creatinine may be used as a safety biomarker when evaluating patients on drugs that affect kidney function to monitor for nephrotoxicity |
| Susceptibility/risk | A biomarker that indicates the potential for developing a disease or medical condition in an individual who does not currently have clinically apparent disease or the medical condition | Apolipoprotein E (APOE) gene variations may be used as susceptibility/risk biomarkers to identify individuals with a predisposition to develop Alzheimer’s disease |

A biomarker is not an evaluation of how an individual feels, functions, or lives', a clinical outcome assessment measure (COA). The Food and Drug Administration - National Institutes of Health (FDANIH) 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].

**Fig 1.** Drug discovery and development processes with potential from biomarkers phases

**III. DISEASE PATHWAY AND POTENTIAL IMPACT OF BIOMARKERS**

Biomarkers are classified into two types: biomarkers of exposure, which are used in risk prediction, and biomarkers of illness, which are used in disease screening, diagnosis, and monitoring.

Biomarkers are widely established in risk prediction, screening, and diagnostic procedures, and they provide distinct and evident advantages. Many neurological illnesses are classified using either established clinical criteria or histology diagnosis. Biomarkers offer the ability to diagnose neurological disease at an early stage, to give a mechanism for homogenous disease categorization, and to further our understanding of disease causation. These benefits are applicable to all forms of clinical research, from clinical trials to epidemiological observational studies [5].

**Disease Pathway**

**Risk factors Screening and Diagnosis Prognosis**

***Induction Latency Disease***

 **Pathogenesis Detection**

**Etiology Biomarkers Disease**

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

A valid biomarker should be:

* a major product of oxidative modification that may be implicated directly in the development of disease;
* a stable product, not susceptible to artefactual induction or loss during storage;
* representative of the balance between oxidative damage generation and clearance (i.e. the steady state, but also possibly applicable to the measurement of cumulative oxidative damage);
* determined by an assay that is specific, sensitive, reproducible and robust;
* free of confounding factors from dietary intake;
* accessible in a target tissue or a valid surrogate tissue such as a leucocyte; and
* measurable within the limits of detection of a reliable analytical procedure [5].

**IV. BIOMARKERS VERSUS CLINICAL ENDPOINT**

By definition, biomarkers are objective, measurable aspects of biological processes. They may or may not correlate with a patient's experience and sense of well-being, and it's possible to conceive quantifiable biological traits that don't correspond to patients' clinical states, or whose fluctuations are undetectable and have no influence on health. It's also easy to conceive observable biological 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 variables that reflect or characterise how a person in a research or clinical trial "feels, functions, or survives." In other words, they are variables that indicate a research subject's health and well-being from the subject's point of view. The purpose of clinical practise is to ameliorate morbidity and mortality, not to modify quantitative characteristics of patients' inherent biochemistry with no obvious clinical consequence, for example. Similarly, individuals want therapy for their ailments, not for numerical indicators that commonly but not always exactly correlate with them. Many consider survival to be the gold-standard clinical endpoint for most HIV trials, but other well-defined, unambiguous clinical variables, such as stroke, myocardial infarction, and the occurrence of predefined opportunistic infections, have also been used as endpoints in appropriate circumstances; they provide clear, unambiguous data that can show definitively whether interventions are effective or ineffective, as well as safe or unsafe. However, not all clinical endpoints are created equal; examples of clinical data items that produce less trustworthy results [6].

**V. BIOMARKERS AS SURROGATE ENDPOINT**

Biomarkers are called surrogate endpoints when employed as clinical trial results; that is, they operate as surrogates or substitutes for clinically significant endpoints. However, not all biomarkers are intended to be surrogate endpoints. Surrogate endpoints are a tiny subset of well-characterized biomarkers having therapeutic use. To be termed a surrogate endpoint, convincing scientific proof (e.g., epidemiological, pharmacological, and/or pathophysiological) showing a biomarker consistently and reliably predicts a clinical result, either benefit or damage, is required. A surrogate endpoint is a biomarker that can be relied on to act as a substitute for, but not as a replacement for, a clinical endpoint [7].

**VI. CHARACTERIZATION AND EVALUATION OF BIOMARKERS**

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Identifying biomarkers as surrogate endpoints necessitates establishing relevance and validity. The potential of a biomarker to give clinically relevant information on issues of interest to the general public, healthcare practitioners, or health policy makers is referred to as relevance. The necessity to describe a biomarker's effectiveness or utility as a surrogate endpoint is referred to as validity. Unfortunately, legitimacy is rarely black and white, but rather a continuum. In fact, some researchers have rejected the word validation as "unsuitable" for the study of biomarkers since it implies a thorough biological explanation of the link between a specific biomarker and a clinical outcome, which they reject [9].

For years, researchers used arrhythmia suppression as a surrogate endpoint for decreased morbidity due to cardiovascular disease, resulting in the approval of anti-arrhythmia drugs (e.g., encainide, flecainide, moricinze) that were later found to increase mortality in certain patient populations in later trials. More recently, a large and well-publicized trial of the combination of two cholesterol-lowering drugs, ezetimibe and simvastatin, highlighted the risk of relying too heavily on biomarkers: while the combination treatment lowered subjects' cholesterol levels more than simvastatin alone, it did not improve atherosclerosis or overall mortality, calling into question much previous research that relied on the assumption that lowering [9].

**VII. BIOMARKERS OF EXPOSURE OR ANTECEDENT BIOMARKERS**

Exposures to the environment, impact modifiers, or risk factors When an illness is suspected of being caused by a hazardous exposure, researchers naturally want to know how much exposure there was. The concentration of the poison in an individual's immediate environment is referred to as external exposure. While surveys provide a historical record of exposure, actual measurement of the suspected toxin in the air, water, soil, or food can provide precise information on the "dose" of exposure. The exterior dose measurement offers the foundation for understanding the link to the illness process, while the "internal" dose measurement may give higher precision. When a toxin is found in tissues or bodily fluids, it serves as a biomarker for the internal dosage. A biomarker that detects a "biologically effective dosage" reveals the quantity of toxin or chemical detected in the target organ or its surrogate. Lead exposure is a great example. Measurement of lead in the environment can reinforce a history of lead exposure, but blood and tissues provide the greatest indication of the dosage of exposure (hair, nails, teeth). Because a variety of bodily fluids might be employed dependent on the pharmacologic qualities of the agent, the pharmacokinetic features of the toxin or chemical of interest become vital to consider when measuring the internal dosage. Some chemicals, like halogenated hydrocarbons, are stored in adipose tissue, but others, like organophosphate insecticides, are best tested in blood or urine [10].

A biomarker of exposure has an advantage over a history of exposure in that it predicts the actual "internal" dosage of the exposure. This enhances precision in risk factor measurement by including both internal and external validity when analysing the influence of exposure on outcome. Because of the pharmacologic features of the chemical or toxin, biomarkers are particularly relevant in the cross-sectional examination of acute illness. Finding biomarkers for exposures that are stable over the lengthy durations necessary for prospective investigations of chronic neurological disorders such as Alzheimer's disease is extremely challenging. Depending on the disease being studied and the pharmacologic properties of the biomarker, banked serum or plasma may be useful in some cases [10].

**Genetic susceptibility**

Using life table methodologies and recurrence risk, epidemiologic analysis may investigate familial aggregation and estimate genetic and environmental factors to an illness. Gene mutations that cause Mendelian illness are often predictable. Variant alleles or polymorphisms in genes may be connected to vulnerability, although they are not deterministic. Most adult-onset degenerative nervous system illnesses are thought to be a combination of heritable and environmental factors. The characteristic or illness is formed by the associated combinations of these traits. As a result, these antecedent biomarkers may or may not be implicated in the pathogenesis. In certain cases, the genetic variation is neither required nor sufficient to induce the disease. They can, however, be potent antecedents at any step of the illness cascade. These antecedent biomarkers, by definition, exist before the disease or result and are unaffected by other exposures. They increase the precision of other associations' measurements since they might be synergistic or antagonistic [11].

**Intermediate biomarkers**

Some biomarkers indicate direct steps in the illness's causative chain and are therefore highly associated to disease. Others are indirectly connected to the cause. There are several options to examine. To produce disease, a biomarker may be dependent on another known or unknown component. As a result, while it is not the lone determinant, it is part of the causation pathway and remains closely linked to the disease. The biomarker might potentially be connected to a previously known exposure or signify a modification produced by the exposure that results in the disease. The most dangerous condition is when the biomarker is linked to an unknown component that is also linked to the exposure [12].

**VIII. ROLE OF BIOMARKERS IN DIAGNOSIS**

Prognosis, screening, and diagnostic tests Biomarkers that represent prodromal signals allow for early diagnosis or the determination of the outcome of interest at a more primitive stage of disease. The biological information required for the diagnosis is provided by blood, urine, and cerebrospinal fluid. Biomarkers are employed as an indication of a biological element that indicates either a subclinical manifestation, stage of the disorder, or a surrogate manifestation of the disease in certain circumstances. Surrogate signs of the illness are frequently represented by biomarkers used for screening or diagnosis.

The potential uses of this class of biomarkers include:

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

2) Reduction in disease heterogeneity in clinical trials or epidemiologic studies,

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

4) Target for a clinical trial. The improvement in validity and precision far outweigh the difficulty in obtaining such tissues from patients [13].

Most ethical review boards and healthcare systems mandate proper follow-up for persons who test positive, whether or not they have the condition. Treatment should also be offered for people who test positive, and it should be accessible and acceptable. Those who test positive and are sick should be given access to therapies, which must be appropriate and readily available. It is important to note that the major advantage of screening is prevention, either primary (before the development of symptoms) or secondary (early or prodromal identification). Consider the advantages of conducting a therapy trial in patients prior to the onset of overt symptoms. Diagnostic tests for neurological illnesses are increasingly being employed in clinical research and practise. The collecting of information from numerous sources, some of which include diagnostic test results, aids in the ultimate objective of enhancing the probability of a certain diagnosis. Clinical tests are also conducted, albeit probably less frequently, for other objectives such as measuring illness severity, predicting disease onset, or monitoring response to a specific therapy. More significantly, illness biomarkers lend themselves easily to clinical studies. Another advantage of this form of diagnostic test is that it reduces disease heterogeneity in clinical trials or observational epidemiologic research, leading to a better knowledge of illness's natural history, which includes the phases of induction, latency, and detection [13].

**Variability**

Although there are various advantages to using biomarkers, unpredictability is a key problem. Variability exists regardless of whether the biomarker is an exposure or effect modifier, a disease surrogate, or an indicator of susceptibility. The quantity of an external exposure or the manner a suspected poison is metabolised can cause interindividual variations. Individuals exposed to the same chemical, for example, may differ in their aptitude (or inability) to metabolise the agent, or they may have had various sorts of exposures (in the field as compared with in the office). Intraindividual variability is typically associated with laboratory mistakes or other variables or exposures that are specific to the individual. Group variability occurs as well, however this is frequently the expected conclusion of a study. Obviously, it works best when group differences are significant. Nonetheless, sensitivity and specificity, or comparable variance estimates, are used to assess a biomarker's capacity to discriminate across groups. Consideration of the sources of variability in biomarker measurement reduces the possibility of exposure misclassification. While measurement error is always an issue with biomarkers, other variables may explain individual or group variability [14].

**Validity**

Precise numbers are appealing, but they are subject to the same issues as any variable. The reliability, validity, sensitivity, specificity, ascertainment bias, and interpretation of data employing biomarkers should be scrutinised in the same way that any other variable is. These issues persist regardless of whether the biomarker is employed as a variable in a clinical trial or an epidemiology investigation. Reliability and repeatability are critical [15].

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

Biomarker is defined as an analyte or measurable disease feature that can be used to objectively quantify the presence, extent, and clinical progress of a disease. An ideal biomarker provides indirect assessment of disease activity and may assist in clinical management. A biomarker should be easily measurable in accessible clinical material. The levels should not vary significantly in the general population. Biomarker levels also should correlate with the disease progression and response to treatment. The preferred biomarkers also should have reliable and rapid measurement. Some of the biomarkers used for screening, diagnosis, and treatment monitoring.

Some of the Biomarkers Used for IEM Disease Biomarker(s)

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

**Biological biomarkers**

Biomarkers of exposure can be divided in two subcategories: internal dose and biologically effective dose. Biomarkers of internal dose aim to determine the compound or its metabolites in tissues or body fluids such as blood, urine, breast milk, and saliva [17].

They can also give information on other sources of exposure to that compound and the existence of genetic polymorphisms for metabolic enzymes. Biomarkers of biologically effective dose assess the interaction of compounds with molecular targets such as DNA and protein receptors (e.g., measurement of DNA and protein adducts in urine and serum). Despite the presence of these adducts being readily measured, DNA adducts have become more popular and one of the most important biomarkers of exposure as their presence may be indicative of the risk associated with the exposure. Although biomarkers of exposure are highly relevant and specific indicators of an exposure, the information given does not necessarily translate into prediction of health consequence, and, therefore, other biomarkers need to be analyzed [17].

**Renal biomarkers**

The number of renal BMs has considerably increased in recent years, expanding from BMs or renal function such as blood urea nitrogen and creatinine, to Bristol Myers Squibb (BMS) of tubular health. These BMs may be assessed in the urine or in the blood. Urinary samples are typically collected overnight on Nalgene mini chiller blocks from animals in metabolism cages and stored frozen at −80°C. Parameters that are frequently evaluated in the urine include α-glutathione S transferase (aGST), kidney injury molecule 1 (KIM1), lipocalin-2 (Lcn2; also named neutrophil gelatinase associated lipocalin), micro-albumin, osteopontin (Spp1), clusterin (Clu), trefoil factor 3 (TFF3), and renal papillary antigen. These can be detected with electro chemiluminescent assays [18].

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

|  |  |  |
| --- | --- | --- |
| *Biomarkers* | *Performance* | *Lesions monitored* |
| K1M1, Clu, Albumin | Many individually outperform and add information to blood urea nitrogen (BUN) and SCr | Acutev kidney tubular alterations in rat |
| Total protein, B2M, Cystatin C | May individually outperform SCr assay and add information to BUN and SCr assays | Early diagnostic biomarkers in rat of acute drug- induced glomerular alterations or damage resulting in impairement of kidney tubular reabsorption |
| TFF3 | Adds information to BUN and SCr assays in rat | Drug- induced acute kidney tubular alterations |

**Hepatic Biomarkers**

The evaluation of the liver function can be done using panels of BM evaluated in the serum. Evaluation for specific types of liver histopathology by ROC analysis led to the identification of parameters considered as relevant markers of hepatic injury. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (Tbili), and serum bile acids (SBA) have the greatest diagnostic utility for manifestations of hepatocellular necrosis and biliary injury, with comparable magnitude of area under the ROC curve and serum hepatobiliary marker changes for both. In the absence of hepatocellular necrosis, ALT increases were observed with biochemical or morphological evidence of cholestasis. After the challenge by hepato-toxicants (CHCl3 and CCl4) the activities of AST and ALT, marker enzymes for liver damage, were elevated remarkably. Serum biomarkers of liver effects are Alanine aminotransferase, Aspartate aminotransferase, Total bilirubin, Serum bile acids, Alkaline phosphatase, Gamma glutamyl transferase, Total cholesterol, Triglycerides, Paraoxonase, Purine nucleotide phosphorylase, Glutamate dehydrogenase, Malate dehydrogenase, miR-122 [19].

**Cardiac Biomarkers**

Major steps forward have been made in the field of cardiac BMs in the past few years, and these have revolved primarily around identifying BMs of myocardial necrosis. This rapid progress in part is the result of the development of ultrasensitive assays for cardiac troponins I (cTnI) and T (cTnT). Cardiac troponins are parts of the contractile apparatus proteins, are specific to the heart, and increases in their serum concentrations were traditionally equated to myocardial necrosis [20].

**Skeletal Muscle**

Biomarkers A variety of drugs, including statins, PPAR-α agonists, steroids, and β-adrenergic receptor agonists, may cause muscle toxicity. Such drug-induced skeletal muscle toxicity may result in serious liability, as illustrated by the withdrawal from the market of cerivastatin (Baycol), the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, after it was associated with 100 rhabdomyolysis-related deaths. Nonclinical detection of skeletal muscle toxicity typically hinges on standard histopathological evaluation, supplemented by the assessment of serological BMs, including 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 before personalised medicine may become a reality in the translation of biomarkers into clinical practise [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 analysed and translated for use in a clinical context. In fact, the hunt for novel 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].

Evaluated in terms of sensitivity, specificity, and predictive ability. False positives and false negatives, as well as positive and negative predictive power, should also be assessed in order to more fully assess the impact of disease misclassification. In a perfect world, the biomarker would have a clear predictive value, but this is not always the case. In particular when other tests are employed, receiver- operator characteristic curves can give the tools required to choose the optimal option in terms of sensitivity and false-positive rates.it's crucial to decide which option will have the best sensitivity and false- rates, especially when other tests are being used. For chronic progressive illnesses, most people positive would agree that screening tests would be ideal. One goal of screening is early detection with the intention of curing the disease completely. Many of the procedures and issues with diagnostic testing also apply to screening. Similar to other diagnostic techniques, sensitivity and specificity provide information about the test's accuracy but not its likelihood of detecting a disease. In order to do that, we must estimate the predictive values (positive and negative) [31].

By increasing the likelihood of disease, diagnostic tests are intended to improve clinical diagnosis, and by definition, the pretest probability would be high. The prior probability is substantially lower for screening, and as a result, the PPV will be lower. Therefore, prevalence, or the likelihood of disease in the past, must also be carefully taken into account during screening. These analytical techniques are currently accessible on numerous statistical software packages. The use of the biomarker in the study must be made known by the researcher. Over interpreting biomarker data is the main cause of errors. For instance, the findings of one study may suggest that a certain biomarker (gathered as a gauge of exposure or susceptibility) is highly linked to a given condition or result. The finding is interpreted by the researcher as a biomarker for the illness or the observed outcome, on the other hand. A biomarker of this kind cannot be anticipated to serve as a diagnostic test until it is a disease manifestation, regardless of how high the odds ratio or relative risk may be. For instance, the APOE-4 allele is significantly linked to Alzheimer's disease, but its existence does not imply the existence of the illness. Some people with an APOE-4 allele do not develop Alzheimer's disease, although many patients without this condition do [31].

**XII. Reference**

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