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# Biomarkers in Medicine: An Overview

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author EF managed the literature and supervised the manuscript preparation. Author SAM-N designed the study. Author RF wrote the protocol and the draft of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

Recently, biomarkers in medicine have gained comprehensive scientific and clinical interest. Biomarker or biological marker defined as alteration in the constituents of tissues or body fluids provide a powerful approach to understanding the spectrum of chronic diseases with application in at least 5 areas like screening, diagnosis, prognostication, prediction of disease recurrence and therapeutic monitoring. Therefore, biomarkers are biological indicators of diseases that can be measured either *in vivo* by biomedical imaging or *in vitro* by laboratory methods. Many kinds of biomarkers are available in the field of medical science with lots of positive as well as negative effect. These markers can also reflect the entire spectrum of disease from the earliest manifestations to the terminal stages and will become one of the major driving forces of pharmaceutical research and drug development in the coming years. Generally, a biomarker is potentially useful along the whole spectrum of the disease process- before diagnosis; for screening and risk assessment, during diagnosis; for staging, grading and selecting the initial therapy and during treatment for monitoring therapy, selecting additional therapy or monitoring recurrent diseases. This brief review describes the types and major uses of biomarkers in clinical investigation.

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## 1. INTRODUCTION

There are several definitions of biomarkers in literature, and they fortunately overlap considerably. In 1998, the National Institutes of Health defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention [1]". The World Health Organization (WHO) has defined a biomarker as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" [2]. Biological markers have been defined by Hulka and colleagues [3] as "cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells or fluids." In practice, biomarkers include tools and technologies that can aid in understanding the prediction, cause, diagnosis, progression, regression or outcome of treatment of disease and risk assessment. These may involve measurements directly on biological media (i.e., amniotic fluid, cerebrospinal fluid, plasma and whole blood, Peritorial fluid, Pleural fluid, saliva, serum, seminal fluid, sweat and urine) [4] or measurements such as brain imaging which do not involve direct sampling of biological media but measure changes in the composition or function of the nervous system [5]. Table 1 shows some types of indicators that are measurable as biomarker in biological media. A biomarker is a parameter that can be chemical, physical or biological [6]. This parameter can be specific cells, molecules, genes, gene products, enzymes, or hormones. The biomarker is either produced by the diseased organ (i.e., tumour) or by the body in response to disease. Biomarkers of all types have been used by generations of epidemiologists, physicians and scientists to study human diseases. Although the term ‘biomarker’ is relatively new, it has have been used in preclinical research and clinical diagnosis for some considerable time [3]. Examples of biomarkers include everything from pulse and blood pressure through basic chemistries to more complex laboratory tests of biological fluids, cells and other tissues [7]. For example, body temperature is a well-known biomarker for fever. Blood pressure is used to determine the risk of stroke. It is also widely known that cholesterol values are a biomarker and risk indicator for coronary and vascular disease and that C-reactive protein (CRP) is a marker for inflammation. Medical signs have a long history of use in clinical practice and biomarkers are merely the most objective, quantifiable medical indicators of modern laboratory science allow us to measure reproducibly [7]. The use of biomarkers and especially laboratory-measured biomarkers, in clinical research is somewhat new and the best approaches to this practice are still being developed [7]. Biomarkers can be used to predict risk for diseases, help screen for diseases, how a patient is likely to respond to a medicine or monitor the patient [8]. A doctor can determine your cholesterol levels to predict your risk for having a heart attack. If your doctor puts you on an anti cholesterol medication, your cholesterol can be measured in a follow-up appointment to determine whether the medication is working; that is whether it has lowered your cholesterol and reduced your risk for having a heart attack. Diabetic patients can test their glucose levels using one test hemoglobin A1C (HbA1c) that estimates glucose levels from the most recent two weeks. Liver function tests (LFT) assess liver toxicity and prostate specific antigen (PSA) assesses prostate cancer risk and disease state. Biomarkers are used in the same way to manage cancers and for other kind of diseases [8,9].

**Table 1. Some types of indicators that is measurable as biomarker in biological media**

| Types of biological media | Name of indicator | Application | More details |
|---|---|---|---|
| **Amniotic fluid (AF)** | Acetylcho-linesterase (AchE) | AChE is primarily active in the central nervous system and small amounts of its found in erythrocytes membrane, skeletal muscle, fetal serum and placenta [61]. Normal amniotic fluid does not contain AChE, unless contributed by the fetus as a result of neural tube defects (NTD). | NTDs are usually identified by ultrasonography and confirmed by AFP assay and AChE electrophoresis in amniotic fluid [62]. |
| | Alpha-fetoprotein (AFP) | Elevated levels of AFP in amniotic fluid long been associated with open neural tube defects and Down's syndrome [62]. | Maternal serum may be used with a combination of tests including AFP for screening for neural tube defects and Down's syndrome in this State [63]. |
| | Sialosyl Tn (STN) and zinccopro-porphyrin-1 (ZnCP-1) | Maternal serum sialosyl Tn (STN) and zinc-coproporphyrin-1 (ZnCP-1) are clinically used as biomarkers for detecting Amniotic Fluid Embolism (AFE). | There is no clear evidence to support the use of STN and ZnCP-1 for the early diagnosis of AFE [64]. |
| **Blood** | Creatinine and BUN | Common biomarkers for kidney injury. | These biomarkers have several disadvantages. Serum levels of creatinine may only change after about 50% of the kidney function has been lost. Therefore, make it impossible to detect acute kidney injury early. Baseline serum creatinine levels vary widely with age, gender, muscle mass, etc. This makes it difficult to determine an accurate standard for kidney morbidity based on serum creatinine. BUN has a similar problem, as it varies with diet and state of hydration [63]. |
| | Serum Amyloid A (SAA) | It is a novel blood biomarker of acute exacerbations of chronic obstructive pulmonary disease (AECOPD) [65]. | Some data indicate that advanced renal cancers are accompanied by increased levels of acute-phase proteins such as SAA [66]. SAA is potentially useful and sensitive marker for early determination of bronchopneumonia and can be used as inflammatory indicator of health in calf herds [67]. |
| | Troponin T and I isoforms | Troponin which is the most sensitive and specific test for myocardial damage, is a superior marker for myocardial injury [18,68]. | These markers are superior to other serum biomarkers of cardiac disease such as creatine kinase (CK)-MB and myoglobin because these proteins are released in patients with skeletal muscle disease or injury, as well as the heart [17]. |

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| **Cerebrospinal fluid (CSF)** | CSF can be tested for the diagnosis of a variety of neurological diseases (subarachnoid hemorrhage and central nervous system infections). | | |
| | Amyloid β42 and Tau protein (total and phosphory-lated) [53] | These proteins as biomarkers can be measured in patients with dementia, to help discriminate between Alzheimer's disease (AD) and other forms of dementia [63]. | The diagnostic guidelines of AD have recently been updated to include brain imaging and CSF biomarkers [69].<br>Diagnosing AD and distinguishing it from other dementias is time consuming and costly, requiring neurological examination, neuroimaging, and blood investigations [70]. Piccini [70], Blennow and Zetterberg [71] indicated that the combination of CSF biomarkers and their ratios may significantly increase the specificity and the accuracy of AD diagnosis.<br>However, currently these tests are applicable to the research setting only and information on how to interpret the tests is limited [63]. |
| | Glucose | Reduction values of CSF glucose are occasionally seen with herpes simplex virus, mumps, some enteroviruses, and lymphocytic choriomeningitis virus [63]. | |
| | Lactate | Increased CSF concentrations are noted in epilepsy and haemorrhage[63]. | |
| | Myelin Basic Protein | This marker may be useful in assessing disease activity in cases of established Multiple Sclerosis [63]. | Myelin basic protein is a major component of myelin. Increased concentrations of myelin in CSF indicate that demyelination is taking place. |
| | Protein | Elevated protein levels may aid in diagnosis of inflammatory conditions such as Guillain Barré Syndrome, where levels of over 1g/L are often seen [63]. | The CSF protein concentration is typically less than 1.5g/L. |
| **Peritoneal fluid** | Amylase | This enzyme raised in pancreatitis [63]. | |
| | Neutrophils | An increase in neutrophils (>250 /μL) is associated with peritonitis (bacterial, tuberculous, pancreatic or malignant) [63]. | |
| | pH | Less than 7.0 indicates bacterial infection [63]. | |
| | Total protein | Greater than 30g/L suggest the fluid is an exudate indicating inflammatory or malignant ascites [63]. | |
| **Pleural fluid** | Adenosine deaminase | This marker has been used to diagnosis of Tuberculosis in the past [63] | In practice this marker regard as a research tool only. |

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| **Saliva** | The functional value of saliva has long been thought to outweigh its diagnostic possibilities. Recent evidence regarding saliva as a diagnostic tool for diseases such as HIV, various forms of cancer, diabetes, arthritis and heart disease has shown that much more information is contained in saliva than was previously thought [72].<br>National Institute of Dental and Craniofacial Research (NIDCR) initiatives and current research efforts are closing the gap rapidly between the use of saliva and other biological fluids (blood, urine, cerebrospinal fluid, tears, and nipple aspirate) for disease diagnostics. Scientific data to establish a benchmark for the diagnostic value of saliva in comparison with that of other biological media will be necessary to assess the disease discriminatory value of saliva. It may well turn out that saliva is more accurate than blood in detecting oral cancer, saliva will outperform other biological media in the diagnosis of other diseases as well [72,73]. | | |
| **Seminal fluid** | Acid phospha-tase | It is used as a marker for the presence of prostatic fluid [63]. | Prostatic acid phosphatase (PAP) is alternative marker to PSA for prostate malignancy. Rape investigations will often include testing for the presence of human prostatic acid phosphatase in vaginal fluid [5]. |
| | Fructose | A very low fructose level in the semen of an azoospermic man indicates absence of the seminal vesicles and/or vasdeferens [63]. | |
| **Sweat** | chloride concentra-tion | Sweat chloride >60mmol/L supports a diagnosis of Cystic Fibrosis (CF). | CF is caused by mutations in a gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein, resulting in dysfunctional epithelial chloride channels [63].<br>Reabsorption of chloride and sodium ions are diminished or abolished in the duct of the sweat gland. Finally, the production of more concentrated sweat with sodium and chloride concentrations of >60 mM [63]. |
| **Urine** | Hematuria | Hematuria is a very general marker and is only present when there is severe kidney injury [63]. | |
| | Pyuria | Pyuria (the presence of white blood cells in urine) is a marker indicating urinary tract infections. | The combined presence of pyuria, proteinuria, hematuria and casts is an indication of glomerulonephritis and nephritic syndrome [63]. |
| | Proteinuria | Proteinuria can occur as a result of glomerular disease, proximal tubule damage, or an excess of serum protein. | Proteinuria is an excess of serum proteins in urine. Proteinuria only takes place after severe kidney damage and takes days to develop after kidney injury has taken place [63].<br>Proteinuria is the most sensitive marker of chronic kidney disease (CKD) progression in clinical practice, especially when combined with estimated glomerular filtration rate(eGFR), but these have limitations [74, 75]. Therefore, new validated biomarkers are required for CKD progression. |

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| Specific gravity (SG) | Elevated/lowered SG of the urine can point to tubular injury, but can also be caused by dehydration [63]. | |
| Casts | Red cell, white cell and epithelial cell casts indicate bleeding into the tubule, inflammation and degeneration of the tubular lumen or necrosis of the renal tubules, respectively [63]. | |
| Crystals | Crystals can be an indicator of inflammation, infection, or a metabolic disorder. | These markers can be influenced by diet and other factors independent of renal damage [63]. |
| pH | It can be an indication of renal failure. | The pH is influenced by sleep and varies before and after a meal. The wide range and dependence on recent activity makes pH difficult to use as a biomarker, but continuous measurements can provide useful information [63]. |
| β2-macroglobulin (β2M) | Increased urinary β2M excretion has been observed to be an early marker of tubular injury in a number of settings including nephrotoxin exposure, cardiac surgery and renal transplantation. | The utility of β2M as a biomarker has been limited by its instability in the urine, with rapid degradation observed at room temperature and in urine with at pH less than 6.0 [63]. Cabre et al. reported that retinol-binding protein 4 (RBP4) was also higher in those individuals with reduced glomerular filtration rate (GFR) and suggested that RBP4 levels could be a marker of kidney dysfunction in diabetic patients [76]. |
| Ceruloplasmin | Urinary ceruloplasmin was found in normoalbuminuric diabetic patients, and its increase in urine had a predictive value for development of microalbuminuria in normoalbuminuric diabetic patients [77]. | All these data suggest that urinary ceruloplasmin is a promising marker of diabetic kidney diseas (DKD), while further studies are needed to characterize its value compared to albuminuria, especially in type 1 diabetics, since all the studies have been done in type 2 diabetics [61]. |
| Cystatin c | Cystatin c, a cysteine protease inhibitor, is a novel biomarker of renal damage [77]. | Serum cystatin c is a good marker for assessing renal injuries, while urinary cystatin c was considered as a useful marker for the detection of DKD [77]. |
| Immunoglobulin G (Ig G) | Urinary IgG excretion correlates with the progression of glomerular diffuse lesions [77]. | |
| Immunoglobulin M (Ig M) | The appearance of IgM in urine indicates that a large, nonselective pore exists in the glomerular capillary wall [77]. | |
| Transferrin | Wang et al. indicated that urinary transferrin excretion was a good marker for predicting onset of nephropathy [77]. | However, urinary transferrin excretion is not specific for DKD because its elevation can be found in primary glomerulonephritis [78]. |

## 2. BIOMARKER'S HISTORY

The idea of using biomarkers to detect disease and improve treatment goes back to the very beginnings of medical treatment. The practice of uroscopy — examining a patient's urine for signs of disease — dates back to the 14th century when doctors would regularly examine the colour and sediment of their patient's urine [6]. In 1960, researchers discovered that some patients with chronic myelogenous leukaemia (CML) have a specific genetic change associated with their cancer, i. e., a shortened version of chromosome 22. This abnormality, known as the Philadelphia chromosome is caused by a translocation between chromosomes 9 and 22. The consequence of this translocation is the creation of the BCR-ABL 'oncogene'. This oncogen produces a protein with elevated tyrosine kinase activity. Researchers were able to use the Philadelphia chromosome as a biomarker to indicate, which patients would benefit from drug candidates (tyrosine kinase inhibitors) [6]. The word biomarker is a little over 30 years old, having first been used by Karpetsky, Humphrey and Levy in the April 1977 edition of the Journal of the National Cancer Institute, where they reported that the "serum RNase level was not a biomarker either for the presence or extent of the plasma cell tumour" [8]. Urine was for many centuries the focus of attention as biomarker because of its easy availability for inspection. Sushruta, the "Father of Ayurvedic Surgery," recorded that the urine of patients with diabetes attracted ants because of its sweetness [8]. However, although the origins of biomarkers are indeed ancient, it is fair to point out that the pace of progress over the first 2500 years was somewhat less than frenetic [10]. One of the most famous biomarker in recent drug development history is the HER-2 gene and receptor, discovered in the mid 1980's. Between 20–30% of breast cancer patients show an over-expression of the HER-2 receptor on their cancer cells [6]. Although this biomarker indicates a higher risk of adverse outcomes, it also gave clinicians a new target for novel therapies [6].

## 3. CHARACTERISTICS OF AN IDEAL BIOMARKER

An ideal biomarker should explain the occurrence of a moderate proportion of the disease in the community. It must have several qualities in order to be clinically applicable. First of all, the biomarker test must be safe and easy to perform. This means that it must be as non-invasive as possible, using external body fluids or blood. The biomarker test should be performed at the bedside or with a (relatively) simple laboratory test using a rapid and reliable standardized platform. Second, a biomarker should be highly specific for the disease and preferably be able to identify subtypes and causes of the disease. Third, a biomarker should be sensitive for as early detection as possible. In addition, the sensitivity and specificity of the biomarker should be relatively high, thus reducing false-positive and false-negative values [6].

## 4. BIOMARKER'S CLASSIFICATION

Biomarkers can be classified based on different parameters [6]. They can be classified based on their characteristics such as:

### 4.1 Imaging Biomarkers: Computerized Axial Tomography (CT), Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI)

Many new biomarkers are being developed that involve imaging technology. Imaging tools can help detect and treat diseases earlier and potentially reduce the financial burden pressuring healthcare today. CT, PET, MRI and nuclear imaging are already widely used in

mainstream imaging and are now expanding into new dimensions [11]. Medical imaging could have a great impact on slow progressing diseases, such as lymphoma, non-small cell lung cancer, Alzheimer's disease and rheumatoid arthritis [12,13]. These markers identified for many diseases of the nervous system, for example, MRI measures in multiple sclerosis and Alzheimer's disease treatments and PET scanning of dopamine transporters in Parkinson's disease, etc [14]. Functional imaging advances provide an understanding of metabolism inside the tumor and its biological pathways by viewing vascular permeability and blood flow. This also allows clinicians to view biological activity at the molecular or cellular level. These can help monitor drug distribution, pharmacokinetics and pharmacodynamics essential for clinical trials conducted early in its lifecycle [6].

Imaging biomarkers have many advantages. They are usually non-invasive, and they produce intuitive, multidimensional results. Yielding both qualitative and quantitative data, they are usually relatively comfortable for patients. When combined with other sources of information, they can be very useful to clinicians seeking to make a diagnosis. Exposing to radiation, high cost and etc are disadvantages of these markers [14].

## 4.2 Molecular Biomarkers

Identification of molecular biomarkers to distinguish physiological conditions or clinical stages is an emerging research field that has grown substantially during the last years. Molecular biomarkers can be used to refer to non-imaging biomarkers, which allow their measurements in biological samples (plasma, serum, cerebrospinal fluid and biopsy) include nucleic acids-based biomarkers such as gene mutations or polymorphisms and quantitative gene expression molecules [6]. Molecular biomarkers are strongly used to monitor treatment, especially in molecular medicine, medical diagnostics, disease prognosis, risk assessment but also in other areas like food safety. There are a number of levels on which molecular biomarkers can be identified, from the beginning of functional protein formation until the deposition of degradation products. Protein formation starts with the encoding of the amino acid sequence on genomic DNA [9]. Epigenetics is an additional field that influences the generation, formation and abundance of mRNA and later proteins by modifying genomic DNA. The versatile transcriptome with all its different components like mRNA, microRNA, short and long non-coding RNAs is the next level on which dynamic changes on the molecular level can occur. The functional proteome itself can be analyzed and at least the metabolites generated can act as potential biomarkers. Another new field in molecular biomarker discovery is the analysis of lipids and their metabolites [9]. Nowadays there are multiple laboratory methodologies available, enabling the analysis of all those putative molecular biomarkers in a high throughput manner. Those methods can be summarized to the ‘‘-omic’’ technologies, namely, genomics, epigenomics, transcriptomics, proteomics, metabolomics and lipidomics. The technological progress in all those ‘‘-omic’’ fields allows the identification of molecular biomarkers in a high number of research areas [9].

Biomarkers based on genetic and molecular biology methods can be classified into three types.

- Type 0- Natural history marker: this type is defined as a marker of disease severity that reflects underlying pathogenetic mechanisms and predicts clinical outcome independent of treatment [15]. The best examples of type 0 markers as independent predictors of risk are the CD4' T-cell count and the HIV 1 plasma RNA level; the former represents a measure of disease severity on the target organ, the immune

system and the latter, a measure of viral burden as an indicator of the activity and extent of infection [15].

- Type 1- Biological activity marker: type I marker is defined as one that responds to therapy; the frequency and magnitude of the response should correlate with the degree of therapeutic potency. Type I marker activity is determined in the context of early phase clinical trials [15]. Triple-drug antiretroviral combinations, for example, appeared superior to single drugs and double-drug combinations in vitro and have been shown in clinical trials to have superior activity with respect to rises in CD4 T-cell counts and declines in HIV 1 plasma RNA levels. In fact, such favorable responses have led to accelerated licensing for many of the currently available antiretroviral agents, including the new class of highly "active" compounds, HIV 1 protease inhibitors. Conversely, absence of type I marker responses, which may be due to an inadequate dose or the lack of promising activity altogether, can accelerate the decision to abort development of a new therapeutic agent [15].
- Type 2: Surrogate marker of therapeutic efficacy: A type II marker, either a single marker or composite of several markers, is defined as one that accounts fully for the efficacy of an agent. The ultimate stage in marker development is to establish the relationship between an early change in the marker and clinical outcome [15]. Ideally, type II markers represent "complete" surrogates of clinical outcome; their most important application is as substitutes for clinical endpoints in phase II/III efficacy studies.

Another category of biomarkers includes those used in decision making in early drug development. For instance, pharmacodynamic (PD) biomarkers are markers of a certain pharmacological response, which are of special interest in dose optimization studies [16]. Biomarkers based on drug development can be described as Diagnostic biomarkers provide the means to define a population with a specific disease. (i.e., cardiac troponin for the diagnosis of myocardial infarction) [17,18].

A prognostic biomarker is a baseline patient or disease characteristic that categorizes individuals by degree of risk for disease occurrence or progression. Prognostic biomarkers informs about the natural history of the disorder in that particular patient in the absence of a therapeutic intervention [9]. Therefore Prognostic biomarkers correlate with outcomes. For example, over expression of Her-2/neu in breast cancer or epidermal growth factor receptor (EGFR) expression in colorectal cancer indicates poor prognoses [6].

A predictive biomarker is a baseline characteristic that categorizes individuals by their likelihood for response to a particular drug treatment. Such a predictive biomarker is used to identify whether a given individual is likely to respond to a treatment intervention in a particular way. It may predict a favorable response or an unfavorable response or adverse event [9]. Some good examples of predictive biomarkers being used in the daily clinical oncology practice are estrogen and progesterone receptors to predict sensitivity to endocrine therapy in breast cancer, Her-2 to predict sensitivity to Herceptin treatment and K-ras mutation to predict resistance to EGFR antibody therapy [6].

### 4.2.1 DNA as biomarker

Increased serum DNA concentrations are associated with various types of cancers and with other diseases such as sepsis and autoimmune disease. Mutations in oncogenes, tumour-suppressor genes and mismatch-repair genes can serve as DNA biomarkers. For instance, there are mutations in the gene that encode the tumour suppressor p53 in more than half of

sporadic cancers [7]. Mutations in other cancer-related genes, such as the *RAS* oncogene, *APC* (the adenomatous polyposis coli gene) and *RB1* (the retinoblastoma gene), also have the potential as markers for prognosis or selection of therapy [19].

### 4.2.2 Circulating DNA as biomarker

When cells first undergo mutations in key target genes and become cancerous, the symptoms do not typically present for months, or even years, imposing serious difficulties for researchers and clinicians to effectively ensure both early and accurate diagnoses. It is acknowledged that effective cancer therapy is often the result of early detection. For this reason and others, the development of reliable methods primarily utilizing non-invasive biomarkers for early-stage cancer detection is of such overriding importance. Thanks to Mandel and Metais [20], the existence of circulating extracellular DNA in the bloodstream was reported as early as 1948. In addition the correlation between cell-free nucleic acid levels in plasma and cancer was initially researched in 1977 by Leon et al [21], who demonstrated for the first time that the plasma levels of free circulating DNA were much higher in cancer patients than in healthy controls. Nevertheless, the mechanisms by which cell-free DNA is released into the bloodstream remain unknown. It has been suggested that lysis of tumor may be the source of the DNA found in plasma/serum of cancer patients [22]. However, the vast majority of reports in the cell-free DNA field indicate that cell death by apoptosis or necrosis could play a role in this phenomenon [22].

### 4.2.3 mRNA as biomarker

The search for mRNA biomarkers is already an established method in different life science fields [9]. In pharmacogenomics it was successfully applied to establish treatment prediction with specific drugs [9]. Hereby the expression of drug sensitive and specific genes was analyzed to predict, if treatment with specific drugs will be promising for the respective individual [23,24]. Using mRNA gene expression analysis is also helpful in the valid differentiation of types or stages of diseases. Thus different forms of heart disease, cancer or neuropsychiatric disorders can be distinguished by analyzing the expression of specific genes [23,24]. In most studies, a number of genes whose expression was influenced by treatment could be identified [9].

### 4.2.4 miRNA as biomarker

miRNAs are small non-coding RNA molecules with about 20–22 nucleotides which are involved in post-transcriptional processing of mRNA. In this way they are able to regulate physiological pathways and metabolic processes [25] and therefore impact the entire cellular physiology, organ development and tissue differentiation. Starting in the late 1990's, a wide array of studies has shown that miRNA levels are differentially expressed in the target tissue during disease or injury. Due to their short length miRNAs are less sensitive to RNase exposure and hence are more stable than the longer mRNA with an average length of 2 kb [26-29]. It holds a unique position among RNA for use as a biomarker due to its unique stability. Studies are showing promise in using miRNA in cell-free body fluids to detect organ injury [30-34]. It has been shown that changes in the spectrum of cellular miRNAs correlate with various physiopathological conditions, including differentiation, inflammation, diabetes and several types of cancers [35]. The hypothesis is that cancerous masses release miRNA into the systemic circulation and therefore, changes in the pattern and amount of miRNA can be used to detect the type of cancer. There exists the hypothesis that tumour cells secrete micro vesicles containing miRNAs into the blood stream and therefore those circulating

miRNAs are very potential biomarkers in the field specific types of cancer diagnostics [32]. For example tissue derived from gastrointestinal cancer can be differentiated from non-gastrointestinal cancer tissue by analyzing specific miRNA profiles [36-39]. Cells undergoing stress may alter the cellular levels of miRNAs and this in turn leads to altered blood levels without any change in the normal processes of miRNA release from the cell. Disturbances in the release processes due to cell stress would directly influence how much miRNA is released into the blood. If cell injury is severe and apoptotic or necrotic death ensues, it is likely that in addition to changes in the normal synthesis and release pathways, miRNAs are also released through the compromised cellular membrane [30]. The alteration of cell-free miRNAs is not restricted to the blood. Weber et al. investigated that Plasma had the highest number of unique miRNA species, followed by saliva. Urine and pleural fluid had no unique miRNA species [35]. Altered levels of miRNAs in a wide array of body fluids such as urine, sputum, feces, bile, cerebrospinal fluid and saliva have been detected in patients suffering various diseases or organ injury such as cancer and alcoholic liver disease [40-44]. For example, Hanke et al. reported that concentrations of 2 specific miRNAs, miR-126 and miR-182, as tumor markers in urine for bladder cancer are changed [45]. Park et al. investigated that miR-125a and miR-200a are differentially expressed in the saliva of the Oral squamous cell carcinoma (OSCC) patients compared with that of healthy controls. These findings suggested that the detection of miRNAs in saliva can be used as a noninvasive and rapid diagnostic tool for the diagnosis of oral cancer [46]. miRNA-based biomarkers also have many advantages over protein-based biomarkers primarily due to the fact that miRNA is a relatively simple molecule that can be detected using standard, robust molecular biology techniques such as real time quantitative polymerase chain reaction (RT-qPCR).

#### 4.2.5 lncRNA as biomarker

Non-coding RNAs with a length of more than 200 nt belong to the group of long non-coding RNAs (lncRNAs). In biomarker research the group of lncRNAs is coming into focus, especially in cancer research. One of the first identified lncRNAs, H19 is a biomarker for tumors of the esophagus, liver, bladder, colon and for metastases in the liver. A loss of methylation in its promoter region leads to a strong up-regulation of this lncRNA, indicating the presence of tumor tissue [47-50].

## 5. BIOMARKERS IN CANCERS

More than 11 million people are diagnosed with cancer every year. It is estimated that there will be 16 million new cases every year by 2020 [51]. Cancer is a cluster of diseases involving alterations in the status and expression of multiple genes that confer a survival advantage and undiminished proliferative potential to somatic or germinal cells [52]. There is increasing evidence to suggest that cancer is also driven by ‘epigenetic changes’ like DNA methylation and altered patterns of histone modifications, leading to alterations in chromatin condensation status thereby regulating expression of certain set of specific genes. Cancer cells display a broad spectrum of genetic alterations that include gene rearrangements, point mutations and gene amplifications, leading to disturbances in molecular pathways regulating cell growth, survival and metastasis [8]. A specific and ideal biomarker for many unbeaten cancer types are still a big challenge. Cancer biomarkers or tumour markers facilitate high-speed, non-invasive cancer diagnosis and enhance early cancer detection and screening. The demand for cancer biomarkers is also increasing because of their ability to trace the exact type of cancer and to target patient-specific molecular structure [8]. These markers can be used to develop targeted therapies, predict risk for cancer, help screen for cancers

and forecast how well a person is likely to respond to a cancer treatment or monitor the patient. Effective tumour markers are in great demand since they have the potential to reduce cancer mortality rates by facilitating diagnosis of cancers at early stages and by helping to individualize treatments [8].

Tumour markers are biochemical substances elaborated by tumour cells either due to the cause or effect of malignant process. These markers can be normal endogenous products that are produced at a greater rate in cancer cells. They may be present as intracellular substances in tissues or may be released into the circulation and appear in serum [53-56].

Detection of high amounts of these markers in blood is suggestive of tumour activity. Serum biomarkers are nonspecific for cancer and can be produced by normal organs as well. One of these serum biomarkers in wide use is PSA [8]. PSA is produced by normal prostate cells in small amounts but higher the PSA in the serum, higher the correlation is towards the existence of prostate cancer. PSA is probably the only serum biomarker currently used consistently in primary care. Cancer antigen 125 (CA-125) can be a biomarker of ovarian cancer risk or an indicator of malignancy, but it has low sensitivity and specificity [8]. Levels of this marker can be high in people who have pancreatitis, kidney or liver disease, making its accuracy as a cancer diagnostic tool very limited [8]. However, it can be used to follow the progress of treatment of cancer and predict a treatment failure when levels rise despite the use of chemotherapeutic agents. Sometimes, a combination of several tumor markers can give risk predictions in someone whose family history for the disease is quite high. Carcinoembryonic antigen (CEA) is another biomarker that is elevated in patients with colorectal, breast, lung, or pancreatic cancer [8]. As a screening test, it can be elevated by many factors other than cancer; smoking for instance raises CEA levels. Following post-surgery CEA levels for colon cancer is an effective way of determining the adequacy of postoperative therapy. While PSA is used in insurance testing to assess the risk of underlying prostate cancer, other biomarkers are neither specific enough nor cost effective to use. Table 2 shows some important cancer antigens that serve as diagnostic and prognostic biomarkers of cancer [57].

Telomeres and telomerase play an important role in the initiation and progression of human cancers [58]. The main function of the telomere is to stabilize the ends of the chromosomes. However, through various mechanisms, telomeres can become dysfunctional, which may drive genomic instability leading to the development of cancer. Because there are significant differences in telomere length and telomerase activity between malignant and non-malignant tissues, many investigations have assessed the potential to utilize these molecular markers for cancer diagnosis [58,59]. Hiyama and his colleague indicated that measurement of telomerase activity or telomerase components has several clinical utilities as a tumor marker: early detection of cancer cells in malignant tumors whose telomerase activity is upregulated in early stages, a prognostic indicator in tumors whose telomerase is activated according to the tumor progression, a marker of malignancy distinguishing from benign tumors and detection of cancer cells in blood [60].

**Table 2. Some types of cancer biomarkers**

| Cancer biomarker | Tumour | Application | More details |
|---|---|---|---|
| Alpha-foetoprotein (AFP) | Hepatocellular carcinoma, Hepatoblastoma | Diagnostic and prognostic | Very significant elevation of serum AFP is documented rarely in malignancies of gastrointestinal tract, pancreas, lungs, kidney, and breast [79,5]. |
| BRCA-1, BRCA-2 | Breast cancer | Diagnostic | BRCA1could be useful as a predictive marker of response to different types of chemotherapy agents such as Anthracyclines [80]. |
| Cancer antigen 125 (CA125) | Epithelial ovarian carcinoma, Fallo-pian tube cancer | Diagnostic and prognostic | CA 125 is the best and the most superior marker today for the epithelial ovarian malignancies [79]. |
| Cancer antigen 15-3 (CA15-3) | Breast cancer | Diagnostic and prognostic | The diagnostic sensitivity of the CA15-3 for breast carcinoma is low as its elevated levels are also observed in benign breast diseases and in liver cirrhosis, acute and chronic hepatitis [79]. |
| Cancer antigen 19-9 (CA 19-9) | Pancreatic cancer Bladder cancer | Diagnostic and prognostic | Non-specific increase of marker is reported in 8% acute and chronic Pancreatitis [79]. |
| Carcinoembryonic antigen (CEA) | Colorectal cancer | Diagnostic and prognostic | CEA assay used for follow-up management of patients with breast, lung, prostatic, pancreatic and ovarian carcinoma [79]. |
| Human chorionic gonadotrophin (hCG) | Germ cell tumours (ovarian Testicular) | Diagnostic | Serum HCG levels are rarely elevated in nontrophoblastic tumors such as lung, breast, pancreas and bladder cancers [79]. |
| Prostate specific antigen (PSA) | Prostate cancer | Diagnostic and prognostic | It is known from many studies that PSA by itself is not a very effective screening tool for the early diagnosis of carcinoma prostate as it is reported to be prostate tissue specific and not prostate cancer specific [79]. However, if combined with digital rectal examination (DRE), Transrectal ultrasound (TRUS), PSA proves very useful in identifying early and curable prostate adenocarcinoma [79,57]. |
| Thyroglobulin (Tg) | Papillary and follicular thyroid cancer | Diagnostic and prognostic | Tg is generally measured in serum but measurements can also be made in thyroid cyst fluids and other fluids/tissue obtained by fine needle biopsy of thyroid nodules [57]. |

## 6. CONCLUSION

Today is the era of technology and every day is day of new invention. Research at present focuses on finding such biomarkers (in human cells) that are linked with specific diseases, and developing assays or sophisticated tests that can detect changes in these biomarkers at very low levels. Since at least the 1980s, the necessity of using biomarkers as surrogate outcomes in large trials of major diseases, such as cancer and heart disease has been widely discussed. The discovery of new disease biomarkers (signatures) and the ability to

measure them rapidly preferably at the initial point of care will revolutionise disease diagnosis. The ability to monitor health status, disease onset and progression and treatment outcome through non-invasive means is a most desirable goal in the health care promotion and delivery. Saliva as a non-invasive specimen is easily collected during a dental visit. It contains oral epithelial cells, microflora and nasopharyngeal discharge as well as a wealth of molecular constituents. Over the past ten years, salivary diagnostics has generated significant interest and attention worldwide, as thousands of salivary proteins, RNA species and metabolites have been identified. Saliva omics studies the biological molecules present in saliva, which encompasses the salivary proteome, transcriptome, microRNA, metabolome and microbiome.

The U.S Food and Drug Administration (FDA) continue to promote the use of biomarkers in basic and clinical research, as well as research on potential new biomarkers to use as surrogates in future trials.

Biomarkers play a critical role in improving the drug development process as well as in the larger biomedical research enterprise. Understanding the relationship between measurable biological processes and clinical outcomes is vital to expanding our arsenal of treatments for all diseases and for deepening our understanding of normal, healthy physiology.

Studies using biomarkers should always have as ultimate measures clinical outcomes, at least for retrospective analysis of biomarker correlation success. We can hope for better tomorrow where early detection will be definitely possible not only for cancer but also for other diseases.

## CONSENT

Not applicable.

## ETHICAL APPROVAL

Not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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