A DEEP DIVE IN TO BIOMARKERS, TYPES, ROLE IN THE DIAGNOSIS OF DISEASES AND IMPACT OF SAMPLE KIND ON SELECTION OF BIOMARKER

Abstract

Biomarkers, also known as biological markers, are biological indicators of the state of a person's biological system. Biomarkers can be used alone or in combination to assess an individual's state of health or disease. A "diagnostic biomarker" is an evaluating tool that can help the physician to determine, confirm, or detect the existence of a medical condition or disease of interest as well as to ascertain whether a victim has an identified disease subtype. These kinds of indicators will become more prevalent considerably as we enter the era of precision medicine. The phrase "monitoring biomarker" refers to a biomarker that is serially assessed for signs of exposure to a medical product or environmental agent, or to test the status of a disease or medical condition to determine the influence of a medical product or biological agent. As a result of exposure of patients to a medication prescribed by a physician or parameter such as environmental factor, "pharmacodynamic/response biomarkers" modify in concentration. Both clinical practice and the initial phases of the development of methods may greatly gain from this particular type of biomarker.

Keywords: Biomarkers, diagnostic biomarker, monitoring biomarker, Pharmacodynamic/response biomarkers, medication, environmental factor.

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I. DIAGNOSTIC BIOMARKERS

Breast cancer is the most widespread malignancy and responsible for the majority of cancer-related mortality in women all over the world, with an estimated 1.7 million incident cases including 521,900 deaths in 2012 [1]. Epidemiological study data has revealed that obesity, advanced maternal age at the time of the first birth, the use of estrogen and progestin, a lack of physical exercise, and alcohol usage are all linked to an elevated likelihood of breast cancer in women [2–5]. Some of these elements have an impact on patients' post-treatment prognoses as well. Genetic variables are crucial to the genesis of breast cancer since individuals with the same circumstances and family aggregation have varying lifetime risks [6, 7]. Gene markers for breast cancer susceptibility, such as BRCA1 and BRCA2, are often employed [8, 9]. Breast cancer is caused by DNA double-strand breaks, which are repaired by tumour suppressor genes. The human genome became unstable as a result of gene mutations, which also elevated the risk of breast cancer by about 21–40% in cases of hereditary breast cancer [10]. [11] developed a graphene-based electrochemical DNA sensor for the detection of BRCA1 using a modified glassy carbon electrode, hybridized target probe DNA, and reporter probe DNA in a sandwich configuration. This sensor was credible, reproducible, and sensitive; it was capable of identifying the BRCA1 gene even at amounts as low as 1 femtomolar. P53 mutations are seen in 30–35% of breast cancers [12]. For the purpose of exploring the p53 gene in detail, a DNA biosensor has been designed [13]. By serially injecting response elements (REs) over the active oligonucleotide probes, the affinities of REs and the p53 gene are shown. These tests show that the affinities of the ligands for the REs differed. A single strand binding protein biosensor was created in order to identify p53 lethal alterations in patients with breast tumors [14]. Breast cancer is found to be associated with exorbitant DNA damage, which is procreated by necrotic and apoptotic cells [15]. Breast cancer treatment information is provided by cell-free tumor DNA (cfDNA) detailed quantification, a novel non-invasive approach to diagnosis. cfDNA has been investigated as a breast cancer biomarker to demonstrate the correlation between cancer development and cfDNA concentration, despite the fact that the technology is not especially advanced. [16,17]. MicroRNAs a group of non-coding RNAs plays a remarkable role in controlling the expression of genes which are now transforming into reliable biological markers based on the hybridization principle and guanine oxidation [18]. Multiple types of electrochemical nanobiosensors have been used to look into the target miRNAs [19]. Despite having the highest sensitivity and specificity of any miRNA marker, MiR-21 has an assortment of shortcomings, notably sequence similarity to related RNAs, appearance in other cancers, and low serum levels. [20]. An acknowledged biomarker for advanced breast cancer, carbohydrate antigen 15-3 (CA15-3), has a relatively low sensitivity for early-stage breast cancer. The mucin MUC1 is detected by CA15-3, which has been widely utilized to identify recurrences and monitor therapy in metastatic breast cancer [21,22,23,24,25]. The typical secretory epithelium's apical membrane contains MUC1, which may be located anywhere along the membrane's outside surface. The clinical value of MUC1 measures is limited to measurements of CA15-3, which is liberated from the cell surface by proteolytic cleavage, despite the fact that MUC1 is expressed in both normal and malignant breast epithelium. About 30% of breast cancer patients had HER2 levels that were much higher than in healthy individuals. Human blood samples can be used to identify HER2, which has been utilized as a breast tumor related antigen [26]. Breast cancer patients often have HER2 levels of 15–75 ng/mL whereas healthy persons typically have values of 2–15 ng/mL [27]. Systemic HER2 levels have been found to be helpful for determining the best course of treatment, such

as delivering Herceptin to patients with HER2 positive breast cancers, and for monitoring disease recurrence and cancer progression [28]. Both disease-free survival and overall survival are dependent prognostic variables for HER2 serum levels, tumor size, nodal involvement, and tumor markers.

1. **Phosphoproteins as Biomarkers – Breast Cancer:** Medical diagnostics has long sought to identify and track illnesses like cancer early through blood testing. Protein phosphorylation, an important post translational modification mechanism and one of the most significant and pervasive molecular regulatory processes, governs literally every aspect of cellular function [29, 30]. Thus, the status of phosphorylation processes may provide a window onto the progression of a disease [31]. Few phosphoproteins, nevertheless, have been created as disease indicators. However, only few phosphoproteins have been established as disease markers. Methods of collecting phosphoproteins from tissues confront substantial obstacles due to the invasive nature of tissue biopsy and the very unpredictable nature of protein phosphorylation throughout the occasionally difficult and drawn-out procedure of tissue biopsy. Furthermore, it is not feasible to evaluate the patient's response to therapy using tumor sample tissue. Developing phosphoproteins into biomarkers of disease from bio fluids is made much more complicated by the elevated levels of active phosphatases seen in blood. Despite certain highly abundant proteins making up over ninety-five percent of the total quantity in blood, very few phosphorylated proteins may be found in steady and measurable levels in plasma/serum. Due to their potential importance in tumor biology and metastasis, extracellular vesicles (EVs), such as microvesicles and exosomes, are currently being investigated to be attractive sources for the development of biomarkers for identifying signs of sickness [32, 33, 34]. Mutations, active miRNAs, and signalling molecules with the capacity for propagation metastatically constitute only a few of the unique traits of the cargo generated by cancer cells [35, 36]. These properties make EVs essential to immune system regulation and intercellular communication. These EV-based markers of disease are an imminent possibility for early-stage cancer and other illnesses since the growing corpus of functional research has demonstrated strong proof that they may be observed long before the emergence of symptoms or physiological detection of a tumor [34, 37]. It is fascinating to learn that EVs are membrane-encapsulated nano- or microparticles that protect their internal contents from external proteases and other enzymes [38, 39, 40]. These features make phosphoproteins in EVs exceptionally stable in a bio fluid for a longer period of time and permit us to synthesize them for use in medical diagnostics. One could be able to obtain more precise real-time information on the biological processes of the organism and the progression of disease, especially in malignancies, by having the capacity to detect the genome output (active proteins, and in particular phosphoproteins).

2. **Biomarker Cerebrospinal Fluid Alpha-Synuclein in the Diagnosis of Parkinson's Disease:** Currently, there are no specific biomarkers to confirm the presence of Parkinson's disease (PD). Alpha α-synuclein was found to be present in the cerebrospinal fluid (CSF) of patients with Parkinson's disease (PD), which is positive but leaves room for doubt. To explore how effectively CSF α-synuclein serves as a diagnostic biomarker of PD and if it can help differentiate Parkinson disease from other neurodegenerative disorders, an intensive literature search of all relevant publications looking for repeated CSF α-synuclein quantification methods in electronic databases was performed. was

carried out. An in-depth review and meta-analysis that was conducted by [41] included a total of 17 trials with 3311 patients. The quantity of CSF α-synuclein concentrations were significantly decreased in PD patients [weighted mean difference (WMD) 0.31; 95% CI, 0.45, 0.16; p 0.0001] as well as Alzheimer's disease (AD) [WMD 0.15; 95% CI, 0.26, 0.04; p 0.0001] compared with normal/neurological controls. Patients with Parkinson's disease (PD), dementia with Lewy bodies (DLB), or multiple system atrophy (MSA) were not significantly different from each other [WMD 0.05; 95% CI, 0.04, 0.13; p = 0.25]. CSF α-synuclein biomarker has showed a sensitivity and specificity of 0.88 (95% CI, 0.84-0.91) and 0.40 (95% CI, 0.35-0.45) for the detection of Parkinson's disease (PD. Based on the CSF concentration of α-synuclein, the odds ratios for the diagnosis of Parkinson's disease were 1.41 (95% CI: 1.24-1.60) and 0.29 (95% CI: 0.15-0.56), respectively. The area under the curve (AUC) for the associated summary receiver operating characteristic (SROC) curve was 0.73. CSF α-synuclein could be considered as a biomarker for the diagnosis of Parkinson's disease [41].

3. **microRNAs as Biomarkers in Heart Failure:** Biomarkers offer an array application in heart failure. They play a significant role in the diagnosis of the ailment and are utilized to figure out the reason behind the cardiac failure. Multiple biomarkers may additionally be employed for foreseeing results and, in specific situations, to guide the choice, effectiveness, and outcome of medication. Finally, biomarkers could assist shed light on some pathophysiological mechanisms that explain heart failure [42]. MiRNAs are fascinating potential emerging biomarkers in heart failure due to the compelling proof tying them to both the beginning and progression of the disease as well as their long-lasting presence in plasma. Circulating miRNAs have undergone extensive research as potential diagnostic biomarkers [43] despite the fact that B-type natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP) are now beginning to be recognized as the gold-standard indicators for excluding and confirming the diagnosis of heart failure, respectively. However, in order to be employed as biomarkers for determining the presence of heart failure, they must either work better than natriuretic peptides or have an additional benefit. Natriuretic peptides have a high degree of sensitivity for the identification of heart failure, although there is still time for improvement. There have been a number of miRNAs suggested as prospective alternatives for heart failure diagnostic biomarkers [44, 45, 46, 47]. Circulating miRNAs were recently used in a few studies to help differentiate between those who encounter dyspnea from other reasons and those who have it because of heart failure. In accordance with an investigation conducted by [48], the level of expression of the gene miR-423-5p differed across those suffering from heart failure, healthy individuals, and patients with various kinds of dyspnea. Acute heart failure is also linked to circulating miRNAs that are unevenly expressed, such as miR-499, which is highly expressed, and miR-103, miR-142-3p, miR-30b, and miR-342-3p, which is weakly expressed [49, 50]. Patients with acute heart failure demonstrated lower levels of these miRNAs when compared with normal control subjects and patients who recently underwent a sudden flare-up of chronic obstructive pulmonary disease [51]. One of numerous miRNAs that were demonstrated to be substantially higher in the plasma of HCM individuals who did not exhibit heart failure symptoms was miR-29a, and it was the only miRNA to be associated with both LV hypertrophy and fibrosis [52]. The results obtained demonstrate that this miRNA may act as a diagnostic tool for the mechanisms involved in HCM remodeling. Additional evidence of miR-29a's specificity to HCM was provided by its ability to discriminate

between hypertrophic obstructive cardiomyopathy (HOCM), hypertrophic non-obstructive cardiomyopathy (HNCM), senile amyloidosis, and aortic stenosis [53]. A significant link was identified between miR-29a and the size of the interventricular septum, a marker for conditions including fibrosis and hypertrophy. MiRNAs may be able to distinguish between heart failure with a preserved ejection fraction (HFpEF) and heart failure with a declining ejection fraction (HFrEF), according to recent studies. Only a small number of circulating miRNAs have been shown to have different levels in HFrEF and HFpEF by three investigations to date [49, 54, 55]. Diagnostically significant differences in miRNA expression between HFpEF and HFrEF can shed light on the unique genesis of each illness.

II. MONITORING BIOMARKER

Hepatocellular carcinoma (HCC) is a most severe form of liver cancer that generally develops in conjunction with cirrhosis and chronic liver disease. It is the fourth cause of cancer-related mortality worldwide and the sixth greatest cause of cancer incidence [56]. While few patients with small, localized HCC may benefit from curative therapies, those who have substantial tumor burden, vascular invasion, or metastasis have a bleak prognosis and are managed with systemic therapy and supportive care. HCC biomarkers are necessary for early detection, prognostication, prediction, and monitoring of responses to therapy. The HCC biomarker that is now extensively employed is alpha-fetoprotein (AFP). The primary HCC screening method advised by leading societies [57, 58, 59] is biannual hepatic ultrasonography with or without serum AFP. In patients with HCC, AFP is employed as a prognostic and predictive biomarker. Increased tumor growth, portal vein thrombosis, waitlist abandonment for liver transplants, and post transplant recurrence have all been linked to elevated levels of AFP [60, 61]. Serum after a liver transplant and ramucirumab therapy, AFP is also a predictor of therapeutic response in HCC patients [62, 63]. However, because to its low sensitivity, AFP has limited utility as a biomarker for the early diagnosis of HCC. When combined with AFP, other protein-based blood tumor indicators including the AFP lectin fraction (AFP-L3) and des-y-carboxy prothrombin (DCP) have been demonstrated to enhance diagnostic efficacy [64]. Despite having been demonstrated to play diagnostic and prognostic roles in HCC, glipican-3 (GPC3) [65], cytokeratin 19 (CK19) [66], golgi protein 73 (GP73) [67], midkine [68], osteopontin [69], squamous cell carcinoma antigen (SCCA) [70], and annexin A2 [71] have not yet been widely incorporated into clinical practice. A liver biopsy enables molecular analysis of the tumor and direct sampling of the tumor tissue. It is an intrusive test, though, and there is a chance of bleeding as well as a worry about potential tumor seeding. Moreover, a single biopsy specimen containing a limited quantity of tumor tissue would not be indicative of the entire HCC tumor since HCCs demonstrate high inter- or intra-tumoral heterogeneity due to genetic abnormalities, transcriptional dysregulation, and epigenetic dysregulation [72]. Many "liquid biopsy" approaches have gained substantial traction in recent years as cutting-edge HCC indicators. Body fluid samples are taken during a liquid biopsy in order to gather crucial phenotypic, genomic, and transcriptomic data on the underlying tumor [73]. The four fundamental elements of liquid biopsies are circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), microRNA (miRNA), and extracellular vesicles (EVs). CTCs are cancerous cells that move into the systemic circulation, either as metastases or from the original tumor, and were first identified in 1869 [74]. Due to the fact that they are a sample of the patient's living tumor cells, CTCs stand out from all other cancer biomarkers [75]. By detecting particular target gene alterations and predicting a patient's

response to or resistance to a certain medication, analysis of CTCs can assist direct treatment strategies.

1. **Biomarkers of Traumatic Brain Injury (TBI):** Traumatic brain injury (TBI) is one of the significant causes of fatalities and disability worldwide, and it is becoming more common among the elderly due to sociodemographic changes [76, 77, 78]. TBI is comprised of two processes: the development of detrimental secondary injuries as a result of the distinctive traumatic impact at the site, that led to primary damage to the brain parenchyma and blood vessels [79], which have been defined by advancing cell death due to inflammation, impaired cerebral blood flow, and impaired metabolic function [80]. Proteins, some of which are extremely concentrated in the CNS, are either produced, released, or leaked by injured, damaged, or dying central nervous system cells [81]. These proteins can be measured in order to determine the degree of cellular damage. The purpose of specialist neurointensive care units (NICUs) that treat TBI patients who are unconscious is to identify, prevent, and treat these secondary insults in order to maximize brain recovery. In clinical practice, measuring these tissue-specific proteins (referred to as "biomarkers") may aid in the early diagnosis of secondary damage [82, 83]. S100B, a calcium-binding protein that is largely intracellular and found in mature, perivascular astrocytes, is the TBI biomarker that has been researched the most [84, 85]. The glycolytic enzyme neuron-specific enolase [86], the astrocytic cytoskeleton component glial fibrillary acidic protein [87], the ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) connected in the neuronal production of ubiquitin [88], and neurofilament light (NF-L) are additional brain-specific proteins that have been thoroughly investigated in TBI. Low serum levels of S100B have been experimentally shown to effectively rule out intracranial injury in patients with mild TBI and reduce the need for head computed tomography in these circumstances. S100B is currently employed locally as an early screening tool in the Scandinavian Guidelines for minor and moderate TBI [89]. The protein's extremely brief serum elimination half-life, however, has been mentioned as one of its drawbacks [90]. Therefore, delayed sampling may be unnecessarily comforting in patients with mild to moderate TBI who lack pathophysiological mechanisms to induce a sustained release in S100B, and this is reflected in the recommendations, which recommend a cut-off of 6 hours after trauma [91]. It is becoming more and more obvious that, in the absence of kinetic factors, a particular serum level is not very significant. How these proteins leave the damaged brain and enter the circulation is not entirely understood. Possible pathways include rupture of the blood-brain barrier (BBB) [92], release independent of BBB integrity [93], and travel through the recently identified glymphatic system [94]. Before being transported to the cerebral spinal fluid (CSF) [96] and/or serum, where samples are easiest to get, these proteins are presumably at first synthesized in the cerebral extracellular space, a site that is challenging to obtain frequently [95]. Numerous factors, including clearance, redistribution, protein stability, and ongoing discharge from the critically injured brain, may have an impact on the availability in serum [97]. Since the kidneys have been shown to completely eliminate the protein S100B, those who were with insufficient renal function may be affected [90, 99, 100]. Given their higher sample sizes and the paucity of research on serum clearance for the other markers, liver metabolism is probably at play [101].

III. PHARMACODYNAMIC BIOMARKERS

Multiple pharmacodynamic signals have been used to identify patients with interferon (IFN) treatment. There is not much evidence to support the use of soluble TRAIL, IP-10, and IL-1RA as pharmacodynamic markers following hypodermic (sc) IFN-1a intake on long-term therapy, despite the fact that some biomarkers, such as neopterin, 2′5 ′-oligoadenylate synthetase, and Myxovirus protein A (MxA), are widely recognized. Biomarker molecules (neopterin, 2′5′OAS, TRAIL, IP-10, IL-1RA) have been investigated in serum samples using affirmed assays. Serum samples from 448 REFLEX trial participants with clinically isolated syndrome (CIS) who received scIFNβ-1a 44g once (ow), three times weekly (tiw), or placebo were taken at baseline (month [M] 0), M6, M12 they came., M18 and M24. At M0 and M24, whole blood MxA gene expression was assessed. In an extension research reflection, 302 people with CIS or individuals who later developed multiple sclerosis (MS) had their blood levels measured every six months for neopterin, IP-10, and TRAIL. The pharmacodynamic effect of each biomarker on adherence to scIFN-1a treatment was assessed using a linear mixed effects model with biomarker expression as the independent variable, biomarker expression at M0, treatment arm, sex, and time as fixed variables and subject as a random effect. Compared to M0, all examined biomarkers significantly increased 1.5–4-fold in response to scIFN-1a treatment. Over the 5-year monitoring period, upregulation vs M0 for each biomarker was sustained and dose-dependent. Patients who received placebo showed no changes, while those who received scIFN-1a 44μg showed intermediate or greater changes. The following pharmacodynamic indicators linked to scIFN-1a therapy were confirmed: neopterin, 2′5′OAS, MxA, IL-1RA, and – on long-term treatment – TRAIL and IP-10 [102].

IV. ROLE OF METABOLOMICS IN BIOMARKER DISCOVERY

The development of disease-modifying or prophylactic drugs relies heavily on the identification of pre-clinical disease biomarkers. The key to effective patient treatment and management is early recognition of the illness. Recent developments in new technologies have led to a surge in studies and activity around the identification of biomarkers. Metabolite changes in biofluids are indicators of physiological or pathological changes. Assessing metabolism in biological systems, both quantitatively and qualitatively, is central to the well-established and rapidly growing scientific topic of metabolomics [103 , 104 , 105 , 106]. The metabolome serves as both the endpoint of the omics cascade and the closest point to the phenotype. As a result, metabolome profiling can be an effective method to identify reliable diagnostic markers to investigate unknown clinical disorders. Metabolomics is a highly effective method for elucidating metabolic pathways that can ultimately contribute to better treatment and diagnosis. It combines phenotype and metabolic signatures, two things that are crucial for biological function [107, 108]. It offers the potential to identify diagnostic markers for therapeutic targets and shed light on the pathophysiology of disease states. The predictive ability of metabolites, which was an advantage of this strategy, performed better in terms of sensitivity and specificity and may be useful for the identification of biomarkers in the future [109]. Moreover, metabolic profiling is very direct, precise and specific and should be equally useful in metabolic research programs.

V. METABOLOMICS IN DIAGNOSIS

Effective treatment of the disease depends on early diagnosis. Identification of disease biomarkers is crucial for early diagnosis, classification, disease progression, prognostic assessment and therapy response. Monitoring the status of living organisms now relies heavily on the analysis of essential metabolites. To understand biochemical changes in linked disorders, metabolomics is a new analytical tool to determine metabolite profiles throughout the body [110]. It is more often used to identify biomarkers for evaluating risk and diagnosing illness [111]. Metabolomics is a relatively young topic in bioinformatics that uses the measurement of metabolite abundance for disease diagnosis and other medical reasons according to recent advances [112]. It shows promise for early diagnosis, expands treatment options, and identifies new metabolic pathways that can be targeted for disorders [113]. Pattern recognition techniques have dominated medical science due to the complexity and volume of data produced by state-of-the-art metabolomics, and may be suitable for some diagnostic medical applications. It is envisioned that the data obtained from metabolite profiling will enable the prescription of personalized drugs that treat the disease more effectively. Metabolome analysis has been used in a number of clinical researches as advances in analytical technology have made it possible to rapidly measure the amount of thousands of metabolites in any biological sample. The deployment of innovative metabolomics tools and associated bioinformatics research have put these goals into sharper focus. Metabolomics, a discipline that is still under development, has enormous potential for clarifying biological mechanisms and locating clinical biomarkers, helping initiatives in improving illness prevention and medical care.

VI. SAMPLE KIND IMPACTS ON BIOMARKER SELECTION

Common diseases can be detected using different sample types and sampling circumstances. For example, urine or swabs can be used as methodologies to collect sample for various sexually transmitted diseases. However obtaining an adequate swab-based sample from male patients may be difficult or uncomfortable for STIs [114, 115]. Therefore, if swab sampling is not feasible for a particular demographic, a well-characterized biomarker may not be effective. In contrast, it is easy to collect urine from all patients in different contexts [116]. It is possible that biomarkers with great clinical sensitivity and specificity, but insufficient concentration to be detected by an efficient analytical approach, will not be helpful in all circumstances. For instance, when urine contains nucleic acid indicators for Chlamydia infection, the clinical concentration of Chlamydia gDNA is only 101 to 106 copies/ml [117]. The amount of Chlamydia gDNA will often be too low to detect if an assay can handle only 100 µl of sample, making gDNA an unreliable biomarker for that test. Despite the fact that gDNA can be amplified, the amount of accessible biomarkers may be limited, which may hinder or restrict biomarker discovery. Collection of high sample volumes would be one way to get around this restriction, but there are limits to how much volume can be obtained without negatively impacting the patient, increasing the signal background, or making the detection assay significantly more difficult. . Moreover, certain situations call for invasive specimens, including tissue biopsies, which are unsuitable for non-trained users to collect. The biomarker of choice can be significantly affected by the type of sample used.

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