The Pivotal Role of Biomarkers in Periodontal and Respiratory Diseases

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“Cultivation of human minds should be the ultimate aim of human existence.”

-Dr. B.R. Ambedkar

CONTENTS

PREFACE

This book explores the pivotal role of biomarkers in understanding and managing periodontal and respiratory diseases, offering a comprehensive examination of their significance in both diagnosis and treatment. Numerous studies have supported the hypothesis that periodontal diseases may impact systemic diseases, including respiratory conditions. With an overarching objective to illuminate the intricate connections between these diseases and biomarkers, it addresses the pressing need for a deeper understanding of their interplay in clinical practice and research. Utilizing a multidisciplinary approach, the methodology employed involves an extensive review of current literature, clinical studies, and cutting-edge research findings in periodontology and respiratory medicine. Key findings highlight the potential of biomarkers as diagnostic tools, prognostic indicators, and targets for therapeutic interventions in both disease domains.

While contributing significantly to the research field, this book also serves as a practical guide for healthcare professionals, as the research landscape is constantly evolving, and new findings may emerge post-publication. Acknowledging the inherent limitations of biomarker research, such as variability in biomarker expression and specificity, as the research landscape is constantly evolving, and new findings may emerge post-publication. The book underscores the need for further validation and standardization efforts. Despite these limitations, the impact of this research is profound, offering novel insights into disease mechanisms and paving the way for personalized treatment approaches.

Contributing significantly to both research and practice, this book bridges the gap between periodontology and respiratory medicine, emphasizing the interconnectedness of oral and systemic health. Its insights have far-reaching implications for clinical management strategies, disease prevention, and the development of targeted therapies, ultimately advancing the frontier of precision medicine in healthcare.

CHAPTER 1

INTRODUCTION TO BIOMARKERS

History

The term "biomarker" originated in the 1970s, with its earliest known usage appearing in an article title in 1973 by Rho et al.[1] Initially, it was employed to signify the presence of material originating from biological sources. This usage persists in geological and ecological literature. The first clinical application of the term can be traced back to 1977 in a publication titled "Tumor biomarkers of value in the management of gynecologic malignancy will also be correlated with clinical course." (Order et al., 1977).[2] However, the concept itself has much older roots, as indicated by references to "biochemical markers" in 1949 (Mundkur)[3] and to "biological 'markers'" in 1957 (Porter).[4]

The term "biomarker," derived from "biological marker," encompasses a diverse subset of medical signs—objective indications of an individual's health observable from an external perspective. These markers are characterized by their ability to be measured with accuracy and reproducibility. In contrast to symptoms, which are experienced subjectively by patients, biomarkers are objective manifestations of health or disease.

Definitions of Biomarker

Numerous precise definitions of biomarkers exist within the literature, often sharing significant overlap. In 1998, the National Institutes of Health Biomarkers Definitions Working Group established a comprehensive definition, describing biomarkers as "characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathological processes, or pharmacologic responses to therapeutic interventions."

This definition underscores the multifaceted nature of biomarkers, emphasizing their utility in understanding and assessing a wide range of physiological and pathological states. From aiding in the diagnosis of diseases to monitoring treatment responses, biomarkers play a crucial role in modern healthcare by providing clinicians with valuable insights into the underlying mechanisms of health and disease.[1]

A joint effort in chemical safety, spearheaded by the World Health Organization (WHO) under the International Programme on Chemical Safety, in collaboration with the United Nations and the International Labor Organization, has provided a definition of biomarkers. According to this initiative, a biomarker is defined 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."This definition highlights the expansive range of biomarkers, including measurable entities within the body as well as external products stemming from bodily processes. Moreover, it underscores the crucial role of biomarkers in offering insights into the onset or progression of outcomes or diseases. Through the identification and quantification of these indicators, researchers and clinicians gain a deeper understanding of health trajectories, enabling the assessment of risks and the implementation of preventive or therapeutic measures. [2]

An even broader perspective on biomarkers considers not only the incidence and outcome of disease but also encompasses the effects of treatments, interventions, and unintended environmental exposures, such as to chemicals or nutrients. In their report on the validity of biomarkers in environmental risk assessment, the World Health Organization (WHO) has asserted that a comprehensive definition of biomarkers includes "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological." The response measured could be functional and physiological, biochemical at the cellular level, or a molecular interaction.This expansive definition highlights the versatility of biomarkers in assessing various interactions between biological systems and potential hazards, whether they stem from chemical, physical, or biological sources.[3]

Examples of biomarkers encompass a wide range of measurements, spanning from basic physiological parameters such as pulse and blood pressure to fundamental biochemical assays and more intricate laboratory tests conducted on blood and other tissues. Medical signs, which have been integral to clinical practice since the inception of medicine itself, serve as the foundation for biomarkers, which represent the most objective and quantifiable manifestations of health and disease that modern laboratory science allows us to consistently measure. While the use of biomarkers, especially those measured in laboratory settings, in clinical research is a relatively newer development, the optimal approaches to this practice are still undergoing refinement. A central challenge lies in establishing the correlation between a given measurable biomarker and relevant clinical endpoints. This process involves rigorous investigation to ascertain the predictive value and clinical significance of biomarkers in guiding diagnostic, prognostic, and therapeutic decisions.

As research in biomarkers progresses, there is a growing recognition of their potential to revolutionize clinical practice by enhancing the accuracy and efficiency of disease detection, monitoring, and management. However, achieving this potential requires ongoing efforts to validate biomarkers, elucidate their underlying mechanisms, and establish robust methodologies for their integration into clinical research and practice.

Comparison of Biomarker vs Clinical Outcomes

Biomarkers, as defined, are objective and quantifiable attributes of biological processes. While they may not always align with a patient's subjective experience of well-being, it's conceivable that certain measurable biological traits exist independently of a patient's clinical state or have variations that are undetectable and inconsequential to health. Additionally, some biological characteristics may exhibit such significant variability among populations that they become unreliable predictors of disease presence or absence.

On the other hand, clinical endpoints refer to variables that reflect how individuals in a study or clinical trial "feel, function, or survive." Essentially, they represent the health and well-being of study subjects from their own perspective. Unlike biomarkers, which focus on biological processes, clinical endpoints directly capture the impact of interventions or conditions on an individual's quality of life and overall health status. [1]

For a considerable time, there has been widespread agreement that clinical endpoints constitute the primary, and for some, the sole relevant objectives of all clinical research, and indeed, of all biomedical research. The overarching aim of clinical practice is to enhance both morbidity and mortality outcomes, rather than simply altering quantifiable aspects of patients' intrinsic biochemistry without any discernible clinical impact. Similarly, individuals seek treatment for their ailments, not solely for the numerical metrics that often, though not always, align with their illnesses.

Biomarkers: Substitutes for Clinical Outcomes

A surrogate endpoint has been defined as ‘a biomarker intended to substitute for a clinical endpoint’, the latter being ‘a characteristic or variable that reflects how a patient feels, functions, or survives’.[4] When utilized as outcomes in clinical trials, biomarkers are categorized as surrogate endpoints, acting as stand-ins or substitutes for clinically meaningful endpoints. However, it's important to note that not all biomarkers serve as surrogate endpoints, nor are they intended. Surrogate endpoints represent a limited subset of well-defined biomarkers with thoroughly evaluated clinical relevance.

For a biomarker to qualify as a surrogate endpoint, robust scientific evidence—such as epidemiological, therapeutic, and/or pathophysiological data—must demonstrate that it consistently and accurately predicts a clinical outcome, whether beneficial or harmful. Essentially, a surrogate endpoint is a biomarker that can be relied upon to represent a clinical endpoint, albeit without replacing it entirely. Even biomarkers that have been statistically validated as surrogates for a specific clinical endpoint may not necessarily be integral to the pathophysiological pathway leading to that endpoint. While there may be instances where evidence suggests that biomarkers measure a process or product of a crucial stage in a pathway, assuming this relationship universally risks conflating correlation with causation. Various explanations exist for biomarkers that only correlate with clinical endpoints under specific circumstances. For instance, multiple interconnected disease pathways may be at play, or the biomarkers could represent indirect indicators of a pathway that isn't fundamentally linked to the key disease processes.[1]

Uses of Biomarkers

Biomarkers, ranging from inflammatory mediators to microbial signatures, serve as invaluable tools for early detection, risk stratification, and monitoring of disease activity. Biomarkers can serve multiple purposes in the field of medicine.[9]

Screen for diseases: Biomarkers can be used to identify individuals who may be at risk for certain diseases or conditions, allowing for early intervention or preventive measures.

Characterize diseases: Biomarkers provide insights into the nature of diseases, including their molecular and physiological characteristics, which can aid in understanding disease mechanisms and developing targeted treatments.

Rule out, diagnose, stage, and monitor diseases: Biomarkers play a crucial role in the diagnostic process, helping to confirm or rule out specific diseases, determine the stage or severity of a condition, and monitor disease progression or response to treatment over time.

Inform prognosis: Biomarkers can provide valuable information about the likely course or outcome of a disease, helping clinicians and patients make informed decisions about treatment and care.

Individualize therapeutic interventions: By monitoring biomarkers, healthcare providers can tailor treatment strategies to individual patients, optimizing efficacy and minimizing potential side effects.

Predict adverse drug reactions: Biomarkers can identify individuals who may be at increased risk of experiencing adverse reactions to certain medications, allowing for personalized drug selection and dosing.

Predict and guide treatment of drug toxicity: Biomarkers can help identify early signs of drug toxicity, enabling timely intervention to prevent or mitigate harm.

Identify cell types: Biomarkers can be used to distinguish between different cell types within tissues or biological samples, aiding in the diagnosis and classification of diseases, as well as in research applications such as histological analysis.

The provided text outlines various criteria and considerations for evaluating the utility and reliability of biomarkers in the context of assessing drug safety and toxicity. Here's a breakdown of each criterion:

Bradford Hill's Guidelines Applied to Biomarkers

The Austin Bradford Hill guidelines are valuable when seeking information to establish a causal association between a biomarker and a clinical disorder. These guidelines provide a structured framework for assessing various factors, such as strength of association, consistency, temporality, biological gradient, specificity, plausibility, coherence, experimental evidence, and analogy. By systematically evaluating these criteria, researchers can determine the likelihood and strength of the causal relationship between a biomarker and a clinical disorder, aiding in the understanding and application of biomarkers in healthcare and clinical practice.

Strength of association: This criterion evaluates how well a biomarker correlates with the exposure level to a chemical and resulting pathology. It involves assessing diagnostic test performances against a gold standard test, ideally in controlled settings such as clinical trials.

Consistency: Consistency examines whether biomarker data remain consistent across different clinical populations and methodological variations. Biomarker multiplexes can enhance consistency, especially in early-stage studies.

Temporality: This criterion emphasizes demonstrating a temporal relationship between biomarker changes and the onset and resolution of pathology after treatment. Prospective studies with carefully selected assessment time points are necessary to establish temporality.

Biological gradient: It refers to a dose-response relationship between the exposure level of a test agent and the severity of biological perturbations and biomarker changes. Testing multiple dose levels can help identify biomarker thresholds for discontinuation of drug treatment.

Specificity: This criterion assesses whether a biomarker changes specifically under the anticipated conditions or under various other clinical conditions. Longitudinal evaluation is necessary to ensure specificity.

Plausibility: Plausibility examines whether there's a biological mechanism linking biomarker changes to observed organ toxicity. Evidence from preclinical studies and molecular, biochemical, genetic, immunological, or physiological associations can support plausibility.

Coherence: Coherence evaluates whether the evidence fits with the known facts regarding the natural history and biology of the outcome. It contrasts with plausibility by rejecting results that contradict existing theories.

Experimental evidence: This criterion considers additional epidemiological and experimental data supporting the biomarker hypothesis, including evidence from drug trials or mechanistic studies.

Analogy: Analogy involves identifying biomarkers or drugs similar to well-described ones associated with a specific endpoint. This can expedite regulatory acceptance. The Bradford Hill approach provides a framework for assessing causal associations between biomarkers, drugs, and organ pathology.

Role of Biomarkers in Periodontal and Respiratory Diseases

Biomarkers encompass a wide range of molecules, including proteins, nucleic acids, and metabolites, which reflect biological processes or disease states in both periodontal and respiratory diseases.

Periodontal Diseases:

Periodontal diseases, including gingivitis and periodontitis, are inflammatory conditions affecting the supporting structures of the teeth. Biomarkers in periodontal diseases can be categorized based on their sources, including host-derived biomarkers, microbial biomarkers, and inflammatory mediators.

Host-Derived Biomarkers: Biomarkers such as matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), interleukins (ILs), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) have been implicated in periodontal tissue destruction and inflammation.[11] For instance, elevated levels of MMPs and decreased levels of TIMPs are associated with collagen degradation and tissue destruction in periodontitis.[12]

Microbial Biomarkers: Certain bacterial species, such as Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia, are commonly associated with periodontal disease progression. These microbial biomarkers, detected through techniques like polymerase chain reaction (PCR) or DNA sequencing, can aid in disease diagnosis and monitoring.[13]

Inflammatory Mediators: Biomarkers like prostaglandin E2 (PGE2), IL-1β, and IL-6 are key mediators of inflammation in periodontal diseases. Their levels correlate with disease severity and treatment outcomes, making them valuable indicators for assessing periodontal health.[14]

Respiratory Diseases:

Respiratory diseases encompass a wide spectrum of conditions affecting the airways and lungs, including asthma, chronic obstructive pulmonary disease (COPD), and pneumonia. Biomarkers in respiratory diseases play crucial roles in disease stratification, predicting exacerbations, and guiding treatment decisions.

Inflammatory Biomarkers: In asthma and COPD, biomarkers such as fractional exhaled nitric oxide (FeNO), eosinophil count, and serum IgE levels reflect airway inflammation and can help differentiate between different disease phenotypes.[15] These biomarkers guide the selection of appropriate therapies, such as corticosteroids or biologics targeting specific inflammatory pathways.

Infectious Biomarkers: In respiratory infections like pneumonia, biomarkers such as procalcitonin (PCT) and C-reactive protein (CRP) aid in distinguishing bacterial from viral etiologies and assessing disease severity.[16] Rapid diagnostic tests based on these biomarkers facilitate timely initiation of appropriate antimicrobial therapy, thereby improving patient outcomes.

Biomarkers of Oxidative Stress: Oxidative stress plays a pivotal role in the pathogenesis of respiratory diseases. Biomarkers such as malondialdehyde (MDA) and 8-isoprostane reflect oxidative damage to lipids and proteins, offering insights into disease progression and response to antioxidant therapies.[17]

The association between periodontal diseases and respiratory conditions has gained attention recently, hinting at a possible link between seemingly unrelated ailments. Studies over the years suggests a bidirectional relationship between them, with evidence of higher prevalence in individuals with one condition also having the other. Chronic inflammation, common to both, is a key factor linking them. Understanding this connection is crucial for comprehensive patient care and highlights the need for interdisciplinary collaboration between dental and respiratory healthcare providers. Biomarkers play a significant role in the diagnosis, prognosis, and management of both periodontal and respiratory diseases.

For instance, in periodontal diseases, biomarkers such as matrix metalloproteinases (MMPs), interleukins (ILs), and prostaglandins in gingival crevicular fluid (GCF) or saliva indicate tissue destruction and inflammation before clinical symptoms manifest.[18] Similarly, in respiratory diseases, biomarkers like exhaled nitric oxide (FeNO) and eosinophils in sputum assist in diagnosing conditions such as asthma and chronic obstructive pulmonary disease (COPD) before the onset of severe symptoms.[19] Biomarkers play a crucial role in monitoring the progression of periodontal and respiratory diseases. In periodontal diseases, biomarkers such as cytokines and enzymes in GCF can track inflammation and tissue destruction over time, aiding in disease management. Similarly, in respiratory diseases, biomarkers like C-reactive protein (CRP) and lung function tests help monitor lung function decline and disease exacerbations, guiding treatment decisions.[20]

Biomarkers help predict how patients will respond to different treatments, allowing for personalized therapeutic approaches. In periodontal diseases, biomarkers can identify individuals who may benefit from adjunctive therapies like systemic antibiotics or anti-inflammatory agents based on their inflammatory profile.[21] Similarly, in respiratory diseases, biomarkers guide the selection of medications such as corticosteroids or bronchodilators, optimizing treatment outcomes.[22] Biomarkers assist in assessing the risk of disease development and predicting prognosis in both periodontal and respiratory diseases. In periodontal diseases, biomarkers associated with systemic conditions like diabetes or cardiovascular diseases indicate increased susceptibility to periodontitis and its complications. In respiratory diseases, biomarkers such as blood eosinophil count or FeNO levels help stratify patients into risk categories and predict future exacerbations or disease complications.[23]

Biomarkers contribute to research aimed at understanding the underlying mechanisms of periodontal and respiratory diseases and developing novel therapeutic interventions. By identifying molecular pathways and targets, biomarkers facilitate the discovery of new treatment modalities and the evaluation of their efficacy in clinical trials.

Conclusion

The exploration of biomarkers represents a pivotal avenue in contemporary biomedical research, offering profound insights into physiological processes, disease mechanisms, and therapeutic interventions. Biomarkers hold immense promise across various domains, including disease diagnosis, prognosis, treatment monitoring, and personalized medicine. The study and application of biomarkers in the context of periodontal and respiratory diseases offer a path for advancing both diagnostic and therapeutic strategies. However, realizing their full potential demands interdisciplinary collaboration, technological innovation, and rigorous validation processes in improving patient outcomes. Thus, biomarkers not only elucidate the complexities of biological systems but also heralds a new era of healthcare innovation and advancement.

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CHAPTER 2

"BIOLOGIC SAMPLES FOR BIOMARKERS"

Introduction

A joint collaborative report on chemical safety, known as the International Program on Chemical Safety, was produced by the World Health Organization (WHO) in partnership with the United Nations and the International Labor Organization. This report defines a biomarker as a "substance, structure, or process that can be measured within an organism's body or its biological formulations, and that influences or predicts the likelihood and incidence of health outcomes or diseases" (WHO International Program on Chemical Safety).[1]

The first acknowledged biological indicator was the Bence–Jones protein, detected as early as 1847 by precipitating a proteinaceous moiety in an acidic and churned urinary sample. Biomarkers are unique to each tissue in the body, reflecting its distinct biomolecular composition. These can include the levels or activities of genes, proteins, or other biomolecules that are crucial for specific bodily functions.[2] They are indicators that are quantified in higher concentrations in various biological fluids, viz., urine, serum, plasma, whole blood, or other bodily tissue sections of patients with various types of tumors.[3,4] Biomarkers encompass a wide range of indicators, including physiological measurements such as pulse rate and blood pressure, as well as tests that range from basic chemical analyses to more advanced laboratory-based evaluations of biological fluids and body tissues.[5]

The ability to measure biomarkers is invaluable in the medical field. By objectively quantifying these biological markers through biochemical assays, researchers and clinicians can assess an organism's exposure to normal or abnormal physiological processes or gauge how effectively it responds to a particular treatment. This capability is especially critical in diagnosing diseases and selecting the most appropriate therapy among several options, ensuring that treatment is tailored to the patient's unique biological profile.

Advancements in omics technologies—such as genomics, epigenetics, metabolomics, transcriptomics, and proteomics—have significantly broadened the scope of biomarker discovery. These cutting-edge techniques enable the identification of novel biomarkers across different biological systems, offering deeper insights into disease mechanisms and patient-specific therapeutic responses. For instance, genomic strategies can reveal genetic variations linked to disease susceptibility, while metabolomics can identify metabolic changes associated with specific pathologies.

In clinical practice, specific biomarkers are routinely used to screen and monitor the health of tissues or serum samples. These biomarkers provide critical information that can guide pharmacological interventions and therapeutic strategies, making them indispensable tools in precision medicine. For example, biomarkers can predict how a patient will respond to a particular drug, thus allowing for more personalized treatment plans.

Biomarkers as a Bioindicators

Biomarkers have long been integral to clinical medicine, and their application has now expanded into new domains, enhancing the early diagnosis and effective treatment of numerous disorders. These biomarkers are divided into five distinct categories based on their relevance to various disease states:

Antecedent biomarkers: These biomarkers are used to identify the risk of disease development, serving as early indicators of potential health issues.

Screening biomarkers: These biomarkers facilitate the detection of sub-clinical disorders, enabling the identification of diseases before they become clinically apparent.

Diagnostic biomarkers: These biomarkers assist in the recognition of overt diseases, playing a crucial role in the accurate diagnosis of existing conditions.

Staging biomarkers: These biomarkers are employed to assess the severity or stage of a disease, helping to categorize the extent of disease progression.

Prognostic biomarkers: These biomarkers provide predictions regarding the prognosis or course of a disease, including the likelihood of recurrence, response to therapeutic interventions, and monitoring the effectiveness of ongoing treatments.[6,7]

Furthermore, biomarkers play a critical role in prognosis, offering insights into the likely course of a disease. They can help predict the chances of recurrence or the expected response to a particular therapy, allowing for more personalized and targeted treatment plans. This ability to tailor treatment to an individual's specific biomarker profile is at the heart of precision medicine, which aims to optimize therapeutic outcomes by considering the unique characteristics of each patient.[8-10]

Biomarkers play a crucial and increasingly significant role in advancing drug development, particularly within biomedical, therapeutic research. Their application has become widespread in both basic research and clinical practice, and their value as key indicators and early endpoints in clinical settings is now widely recognized across nearly all research domains.[11,12]

Classification of Biomarkers

The clinical value of biomarkers, regardless of their nature, is primarily determined by their sensitivity, specificity, predictive accuracy, and the precision with which they can be measured. Additionally, their reliability, reproducibility, and potential for broad applicability are essential factors. For biomarkers to be successful, they must undergo rigorous validation processes, tailored to the level and context of their application. It is critical that biomarkers possess specific characteristics suited to their intended purpose, ensuring that they meet the stringent requirements for sensitivity, accuracy, and precision. This is necessary for their results to be consistently reproducible in the analyses for which they are designed.[13]

The evolution of regulatory guidelines for the application of biomarkers is critically important and relies on the thorough conduct and well-defined evaluation of biomarker assessments. These guidelines establish the criteria that facilitate the translation of research findings into clinical practice, enabling evidence-based data to support the clinical applicability of novel biomolecules.

Biomolecules can be categorized based on the method of information acquisition into the following types:

Biochemistry or Histology-based Parameters: These biomarkers are detected in tissues obtained through biopsy procedures or surgeries, focusing on biochemical or histological characteristics.

Biochemical Indicators of Cells: These biomarkers are derived from biological fluids and reflect cellular biochemical changes or conditions.

Anatomical, Molecular, or Functional Features: These biomarkers are demonstrated using bio-imaging technologies, which reveal structural, molecular, or functional aspects of the body.

Biomarkers possess specific characteristics that can be objectively quantified and evaluated as indicators of normal biological processes, pathological conditions, or the pharmacological effects of various drug therapies. These are categorized into:

GENE BASED: Nuclear DNA, Mitochondrial DNA, RNA, mRNA, MicroRNA

PROTEIN BASED: Protein, peptides, Antibodies

METABOLISM BASED: Lipids, Carbohydrates, Enzymes, Metabolites

SOURCE BASED: Blood, Serum, Plasma, Tissues

DISEASE BASED: Prediction biomarkers, Detection biomarkers, Diagnostic biomarkers, Prognostic biomarkers.

OTHER: Imaging biomarkers, In-silico biomarkers, Pathological biomarkers

These biomarkers can be established through both biochemical and clinical validation, and while they can be correlated with certain disorders, they are not necessarily causally linked to all disease mechanisms. Depending on their application, these biomarkers can function as diagnostic tools, indicators of disease prognosis, or as classifiers for different disease types.[14,15]

Pharmacokinetic or monitoring biomarkers are measurable indicators used to evaluate the status of a disease or medical condition by detecting exposure to a medical or environmental agent or by monitoring the effects of a medical or biological agent. These biomarkers are crucial in assessing the distribution of a drug to specific or targeted locations, determining the residence time of the drug at its target, and evaluating how the drug interacts with or alters its targets. They reflect the concentrations of pharmacological drugs in the blood or other body fluids, both circulating and at the sites of pharmacological action, and are essential for calculating the appropriate doses needed to induce desired pharmacological responses.[16,17]

Pharmacodynamic biomarkers are those that change in response to exposure to a medical or environmental agent, serving as indicators of the effects of these exposures. These biomarkers demonstrate the functional consequences of a drug's interaction with its targets, often referred to as pharmacological bioindicators, and can be measured using various techniques such as enzymology, imaging, and omics. They are particularly valuable in evaluating the therapeutic efficacy of treatments and in identifying potential adverse drug reactions, thus aiding in the rationalization of clinical therapies.[18,19]

A predictive biomarker indicates that the presence or alteration of the biomarker can forecast an individual's or population's exposure to a medical or environmental agent. These biomarkers are valuable for predicting which patients are likely to respond to a specific therapeutic regimen or drug mechanism, as well as for identifying those who may experience adverse drug reactions.[20,21]

A surrogate endpoint is a type of biomarker intended to substitute for clinical outcomes. These biomarkers are expected to predict clinical benefits, harms, or lack thereof, based on epidemiological, therapeutic, pathophysiological, and other scientific evidence.[22,23]

Validated biomarkers are those that can be accurately measured using analytical testing systems with well-defined performance characteristics. These biomarkers are supported by a robust scientific framework and a comprehensive body of evidence that clarifies the physiological, pharmacological, toxicological, or clinical significance of the test results.

Specimens Useful for Measuring Biomarkers

Non-invasive as opposed to invasive methods should be used whenever possible

Noninvasive samples.

Type of tissue	Mode of collection
Buccal epithelia	Swab of inner lining of cheek with tongue depressor or cytobrush
Saliva	Sterile plastic pipette or specially prepared cotton swab
Urine and urothelial cells	Separated by centrifugation
Nasal epithelia	Swab of inner lining of the nose with cytobrush or cotton swab

Expired air	Spirometer attachment
Hair	In container after cut or fallen out
Fingernails	Clippings in sterile container
Extracted teeth	Collected in sterile container after loss
Dental plaque	Collected with explorer

Invasive methods

Types of tissue	Mode of collection
Blood , serum	Venipuncture or finger prick
Bronchial, esophageal, GI tract epithelia	Biopsy material
Bone marrow	Spinal tap
Amniotic fluid	Amniocentesis (Mother)
Adipose tissue	Biopsy
Gingiva	Excisional or incisional biopsy
Gingival crevicular fluid	With the help of paper points
Broncho alveolar lavage fluid	Bronchoalveolar lavage

Sample Collection

Non-invasive sample collection methods, such as gathering exfoliated cells from the mouth (buccal) or urine (urothelial), offer significant advantages primarily by enhancing participant recruitment and retention. The ease and minimal discomfort associated with these methods make them more appealing to participants, who are generally more willing to undergo a simple buccal swab than a more invasive procedure like a blood draw or biopsy.

Furthermore, non-invasive sample collection simplifies logistical aspects of study design. For instance, procedures like blood draws or biopsies require the presence of highly trained medical personnel, specialized equipment, and sterile environments. In contrast, collecting buccal or urothelial samples can be done with minimal training, often by the participants themselves. This self-collection capability is particularly valuable in remote or underserved areas where access to healthcare facilities and trained personnel is limited.

Safety concerns are paramount when handling biological samples, necessitating thorough training for all researchers and staff involved in a study. Proper handling procedures are essential to ensure the safety of those directly working with the samples, as well as others who may come into contact with them. The risk of exposure increases significantly in studies related to infectious diseases, particularly in international research conducted in regions with high prevalence rates of such diseases. Therefore, strict adherence to safety protocols is crucial to minimizing potential hazards. Extra care must be taken when sample collection involves needles or other sharps.

The instability of certain biomarkers necessitates careful consideration of factors such as processing timing, temperature control, the use of stabilizing chemicals and buffers, and maintaining sterility. Adhering to established protocols is critical, as it reduces variability in biomarker stability between samples and enhances the reliability of biomarker assessments within each sample. Additionally, the choice of anticoagulants and stabilizing agents should be thoroughly evaluated and tested before being implemented in larger-scale research to ensure their effectiveness and minimize potential issues.

The timing between the collection and processing of a biological sample is a critical factor that can significantly impact the integrity and reliability of the data obtained. Different types of analyses and experiments have varying sensitivities to delays between collection and processing, making it essential to tailor the handling procedures to the specific requirements of the study.

To ensure the accuracy and consistency of time-sensitive analyses, it is imperative to have a well-coordinated workflow that minimizes the time between collection and processing. This may involve having the necessary equipment and personnel on hand, as well as pre-established protocols that allow for the rapid initiation of the processing steps.[26] To ensure the integrity of these samples, it is crucial to control the temperature between collection and processing, as well as during storage, to preserve their stability.[27]

Beyond preserving the biological components in collected samples, it is essential to ensure that the measurement of environmental pollutants within the samples is not compromised by contaminants from the containers or tools used during collection. Depending on the specific toxicant being studied, it may be necessary to prescreen these materials to prevent contamination and ensure accurate analysis.

Quality Assurence

Good Laboratory Practices (GLP) and Quality Assurance/Quality Control (QA/QC) programs cover all aspects of laboratory operations, from ensuring equipment is properly calibrated to maintaining raw data integrity. It is essential for every laboratory to implement a comprehensive program that enforces adherence to GLP and QA/QC procedures to ensure consistent and reliable results. Documentation for QA/ QC programs is provided by the EPA at http://www.epa. gov/quality/qatools.html

Sample Processing

The processing of biological samples such as blood, buccal cells, and urine generates multiple types of specimens that can be either analyzed promptly or banked for subsequent research. A range of established protocols exists to prepare these samples for biomarker analysis, each designed to optimize the quality and integrity of the samples according to the specific requirements of the study.[28]

In situations where the collection site is not in close proximity to a laboratory, certain basic processing steps can be performed on-site to maintain sample quality. These steps might include aliquoting (dividing the sample into smaller portions), separating serum from clots in blood samples, and rapidly freezing the samples at -80°C. These procedures help preserve the biological components of the samples, minimizing degradation during transportation to a laboratory for further analysis.

However, many more complex processes require the controlled environment of a well-equipped laboratory. Such processes often involve sterile techniques, specialized reagents, and sophisticated equipment to ensure that the samples are processed accurately and without contamination. These procedures might include detailed biomarker assays, molecular analyses, or cell culture work, which demand a higher level of precision and control than can typically be achieved in a field setting.[27]

Biologic Samples for Periodontal Diseases

A variety of biological samples have been explored as potential sources of biomarkers for diagnosing periodontal diseases. Saliva is frequently used as it provides valuable information about both local oral conditions and systemic health. Gingival crevicular fluid (GCF) is particularly specific for