BIOMARKERS IN MEDICINE

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ISBN (Online): 978-981-5040-46-3

ISBN (Print): 978-981-5040-47-0

ISBN (Paperback): 978-981-5040-48-7

First published in 2022.

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CONTENTS

PREFACE

"Basically, there are two things in life, science and personal thoughts. The first leads to knowledge, the second to ignorance (Hippocrates)."

This detailed book will provide a vital guide to further understanding the current and future biomarkers, one of the most important trends in healthcare today. This book will facilitate the combination of therapeutics with diagnostics and will consequently feature the key correlations between diseases and classifications of biomarkers. The book has chapters on topics such as cancer, neuroscience, cardiology, immunology/immunotherapy, metabolism, pharmacology, haematology, obstetrics and gynecology, hepatology, aging, obesity, urology, nephrology, microbiology, gastroenterology, pediatrics, surgery, pulmonary diseases, pathology and also emphasizes on the preclinical and clinical manifestation of the injury and disease process. This book will supply the unique insight of an expert with extensive experience in diagnostics and clinical laboratory, offering case studies and practical examples from different classes of biomarkers on different platforms, including new data for biomarkers in different therapeutic indications. Clinicians managing patients or clinical trials, clinical researchers, clinical laboratories, diagnostic companies, regulatory agencies, medical school graduate students, academic students, and the general public will all benefit from this book. We would like to thank all my colleagues, Bentham publishing house, and the staff for their support in the preparation of this book. A good book is a real treasure.

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CHAPTER 1

New Biomarkers and Immunotherapy Decision

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Abstract: As immune checkpoint blockade and other immune-based therapy approaches lead to broad treatment advances among patients with advanced cancer, an important consideration is how to best select patients whose tumors will respond to these therapies. As a consequence predictive and prognostic markers are needed. There are genomic features, such as tumour mutation burden (TMB), microsatellite instability (MSI), and immune phenotype features, such as programmed death-ligand 1 (PD-L1), CTLA-4 and tumour infiltrating lymphocytes (TILs), to predict response to immunotherapies (ITs). Several studies show the correlation between TMB and predicted neoantigen load across multiple cancer types. Response to immune checkpoint inhibitors is higher in tumours with high TMB. The candidate biomarker that has been studied mostly other than TMB is PD-L1 expression in trials utilizing programmed cell death-1 (PD-1) blockade. PD-L1 and PD-1 expression are dynamic markers that change in relation to local cytokines and other factors, and the thresholds that separate "positive" and "negative" PD-L1 expressions remain under debate. PD-L1 expression is now a routine diagnostic marker for patients with newly diagnosed NSCLC. The potential applicability of PD-L1 in other disease settings is still uncertain. Microsatellite instability is characterised by high rates of alterations to repetitive DNA sequences caused by impaired mismatch repair (MMR); MSI was the biomarker was approved according to tumor's initial location. Combining TMB with specific genomic alterations is crucial. Moreover, new biomarkers are being investigated.

Keywords: Checkpoint inhibitor, Immunotherapy, MSI, PD-L1, PD1, Predictive , Prognostic , TIL, TMB.

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Pēnar Atukeren and Hafize Uzun (Eds.)

NEW BIOMARKERS AND IMMUNOTHERAPY DECISION

Principles of Cancer İmmunotherapy

Immunotherapy (IT) is a type of biological therapy. Biological therapy is a type of treatment that uses cytokines made from living organisms to treat cancer. What is expected is that the immune system detects and destroys the abnormal cells and likely inhibits the growth of many cancers. For example, immune cells are sometimes found in and around tumors. These cells, called tumor-infiltrating lymphocytes or TILs, are a sign that the immune system is responding to the tumor. People whose tumors contain TIL generally do better than people who do not have tumors [1].

Even though the immune system can prevent or slow cancer growth, cancer cells have ways to avoid destruction mediated by the immune system. Cancer cells may have genetic changes that make them less visible to the immune system; they also have proteins on their surface that turn off immune cells and change the normal cells around the tumor, thus interfering with how the immune system responds to the cancer cells [2]. Immunotherapy helps the immune system to act better against cancer.

Tumor Immunology

Cell types involved in tumor recognition and rejection — An effective and specific cytotoxic immune response against a tumor requires a complex, serially evolving interaction between various immune cell types in the adaptive and innate immune systems. The Th1/Th2 subclasses of CD8+ lymphocytes and CD4+ T lymphocytes are conventionally referred to as cytotoxic T cells and helper T cells. CD8+ and CD4+ lymphocytes initiate the distinction between self and non-self antigens through recognition at the “immune synapse.” Natural killer (NK) cells do not require antigen presentation by the major histocompatibility complex (MHC) for cytotoxic activity. In fact, NK cells target cells with low MHC class 1 expression for degradation. Like T cells, NK cells express numerous inhibitory molecules, notably the very different lethal immunoglobulin-like receptor (KIR) subtypes [3].

Additional cell types, such as FoxP3+, CD25+, CD4+, T regulatory (Treg), and myeloid-derived suppressor cells (MDSCs), largely inhibit cytotoxic T lymphocyte activity [4, 5]. Th17 cells, subsets of CD4+ T cells that secrete interleukin (IL)-17, are implicated in autoimmunity and cancer [6]. Macrophages differentiate into at least two different phenotypes: M1 macrophages, which release interferon (IFN) gamma and are responsible for phagocytosis, and M2

macrophages, which release cytokines, such as IL-4, IL-10, transforming growth factor-beta (TGF-beta), and dampen inflammatory responses and foster tolerance [7]. The "immune synapse," the most widely studied phenomenon in immunologic surveillance, is the ability of T lymphocytes to distinguish self *versus* non-self antigens, which are presented by antigen-presenting cells (APCs) such as dendritic cells. Overall, the cytotoxic activity of a CD8+ T cell is regulated by the presence and spatial orientation of a set of stimulatory and inhibitory receptors whose expression is regulated by a myriad of cytokines. Together, this configuration is often referred to as the "immune synapse."

Therapeutic Approaches

A number of therapeutic approaches are being studied to unleash the immune system and control malignancy. These approaches include cytokines, T cells (checkpoint inhibitors, agonism of costimulatory receptors), manipulation of T cells, oncolytic viruses, therapies directed at other cell types, and vaccines.

Cytokines — Early approaches to immunotherapy exploited the numerous diverse effects of cytokines and other substances that affect immune cell activity. Examples include Interleukin (IL)-2, which was originally discovered as a T cell growth factor. IL-2 has pleiotropic effects on both cytotoxic T cell function and T-regulatory (Treg) cell maintenance. Effects vary in part with the dose and timing of IL-2 administration [8]. Although IL-2 use has been largely supplanted by the use of checkpoint inhibitors, bolus high-dose IL-2 achieved durable objective responses in a minority of patients with melanoma and renal cell carcinoma (RCC), serving as proof of principle that the immune system could eliminate cancer cells [9, 10].

Immune Checkpoint Inhibitors (ICI)

1) PD-1 and PD Ligand 1/2: Programmed cell death 1 (PD-1) is a transmembrane protein expressed on T cells, B cells, and NK cells. It is an inhibitory molecule that binds to the PD-1 ligand (PD-L1; also known as B7-H1) and PD-L2 (B7-H2). PD-L1 is expressed on the surface of multiple tissue types, including many tumor cells, as well as hematopoietic cells; PD-L2 is more restricted to hematopoietic cells. The PD-1:PD-L1/2 interaction directly inhibits apoptosis of the tumor cell, promotes peripheral T effector cell exhaustion, and promotes the conversion of T effector cells to Treg cells [11, 12]. Based upon prolonged overall survival in phase III trials and durable responses in phase II studies, antibodies inhibiting PD-1 (pembrolizumab, nivolumab) and PD-L1 (atezolizumab, avelumab, durvalumab) have been approved for a number of clinical indications and are being evaluated in multiple other malignancies; these are discussed in specific disease-related topics.

CHAPTER 2

Biomarkers in Gynecologic Tumors

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Abstract: Gynecologic malignancies are one of the most frequent cancers amongst women. Biomarkers are crucial for the differential diagnosis of adnexal masses; however, their potential for diagnosis is limited. In the era of difficulty in ovarian cancer screening, novel biomarkers are defined, but CA125 still remains the most valuable one. Circulating tumor DNAs, DNA hypermethylation, metabolites, microRNAs, and kallikreins have recently turned out as ovarian cancer biomarkers and are being applied to clinical practice. For uterine cancer, genomic classification has now been described, it will be used as a prognostic tool. In this chapter, we describe ovarian, endometrial, and cervical cancer biomarkers in detail.

Keywords: Biomarker, CA125, Cervical cancer, cfDNA, HE4, High-copy number, miRNA, MSI, MSS, Ovarian cancer, POLE-mutated, ROMA, SCC-Ag, Uterine cancer.

INTRODUCTION

The lifetime risk of developing ovarian cancer (OC) is approximately 1.3% in women, and OC is the most common cause of gynecological cancer deaths [1]. OC is a generalized term for tumors that involve the ovary, and it can be classified into three different subgroups: epithelial, germ cell, and specialized stromal cell tumors. The most common form of ovarian cancer is epithelial ovarian cancer (EOC), which consists of four main histologic subtypes: serous, endometrioid, clear cell, and mucinous cancer. Serous ovarian carcinoma is the most common form [2].

Ovarian and Fallopian Tube Cancers

Carbohydrate Antigen 125 (CA125)

CA125 is a mucin-type glycoprotein that is associated with the cell membrane,

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Pınar Atukeren and Hafize Uzun (Eds.)

and it is recognized by the OC125 murine monoclonal antibody. CA125 is not only expressed in the tubal, endometrial, and endocervical epithelium but also mesothelial cells of the pleura, pericardium, and peritoneum [3 - 5]. Hence, CA125 is not a specific biomarker for OC.

The cutoff value of 35 U/mL is used in routine clinical practice in premenopausal women, but levels of CA125 tend to be lower in postmenopausal women, so a cutoff value of 26 U/mL has been suggested [4, 6]. Elevated serum levels of CA125 can be detected in approximately 85% of patients with EOC, especially in serous type, but also in benign and physiological states such as fibroids, endometriosis, menstruation, and pregnancy as well as in other malignancies such as pancreas, liver, colorectal and breast cancer (Table **1**) [7 - 9].

Screening

Screening modalities for OC are primarily focused on CA125 and the use of transvaginal ultrasonography (TVU). Early detection of OC that requires a screening test with high sensitivity (>75%) and ultrahigh specificity (99.7%) could improve overall survival [10]. False positivity for CA125 has been shown approximately in 1% of the healthy population and 5% of patients with benign disease that limits its use as a stand-alone test [4, 11].

Table 1. CA125 elevations in gynecologic diseases and non-gynecologic malignancies.

Disease Category	Type of Disease	CA125 > 35 u/ML (%)
Benign gynecologic	Ovarian Cysts[a]	14
	Abscess/Hydrosalpinx/PID[a]	37
	Endometriosis/Endometriomas[a]	67
	Fibroid (Leiomyomas)[a]	26
	Cystadenoma, Adenofibroma, Cystadenofibroma[a]	20
	Serous Epithelial Tumors[a]	20
	Mucinous Epithelial Tumors[a]	18
	Germ Cell Tumors (Mature Teratoma)[a]	21
	Sex Cord Stromal Tumors (Thecoma, Fibrothecoma)[a]	52
Ovarian cancer[b]	Serous[b]	80
	Mucinous[b]	69
	Endometriod[b]	75
	Clear cell[b]	78

(Table 1) cont.....

Disease Category	Type of Disease	CA125 > 35 u/ML (%)
Gynecologic cancers[b,c]	Endometrial[b]	25
	Cervical[c]	39
Non-gynecologic cancers[d]	Pancreas[d]	52.6
	Liver[d]	49.0
	Biliary Tract[d]	45.8
	Colorectal[d]	15.1
	Breast[d]	17.6

[a]Moore RG, Miller MC, Steinhoff MM, *et al*. Serum HE4 levels are less frequently elevated than CA125 in women with benign gynecologic disorders. Am J Obstet Gynecol 2012; 206(4):351. [b]Jacobs I, Bast RC Jr. The CA 125 tumour-associated antigen: a review of the literature. Hum Reprod 1989; 4:1-12. [c]Muyldermans M, Cornillie FJ, Koninckx PR. CA125 and endometriosis. Hum Reprod Update 1999; 1(2):173-87. [d]Bast RC Jr, Klug TL, St John E, *et al*. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 1983; 309:883-7.

The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial enrolled 78,216 women aged 55-74 years, with 39,105 women randomized annual screening. Women were screened annually with CA-125 for 6 years and TVU for 4 years. Neither at a median follow-up of 12.4 years nor at an extended follow-up of 14,7 years, the PLCO trial reported reduction in mortality rates [12, 13]. On the contrary, the false positivity in the PLCO trial was approximately 5%, resulting in complicated surgeries [12].

In the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS), 202,638 postmenopausal women aged 50-74 were randomized to either control or annually multimodal screening group (MMS) or ultrasonography screening (USS) group in a 2:1:1 ratio [14]. Although the trial reported an insignificant mortality reduction on primary analysis (15% in the MMS group, 11% in the USS group), further analysis revealed that a declined mortality was seen after seven years of follow-up (23% in the MMS group, 21% in the USS group) [14].

Hereditary breast-ovarian cancer (HBOC) syndrome, which is related to mutations in BRCA1 and BRCA2 genes, accounts for 14% of EOC. Other identified genes (Lynch syndrome, RAD51C, RAD51D, and BRIP1) account for a minor percentage of EOC [15]. The average risk of developing OC is around 44-45% for BRCA1-mutated women and 12-17% for BRCA2-mutated women [16, 17]. Serous ovarian cancer is the most common histologic subtype for both BRCA mutation carriers [18].

The lifetime risk of endometrial cancer in Lynch syndrome (LS) carriers is 40% due to mutations in mismatch repair genes (MLH1, MSH2, MSH6, and PMS2)

CHAPTER 3

Biomarkers in Urological Cancers

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Abstract: Urological tumours have become one of the most common cancers in the last decade. It is important to apply an approach that evaluates many factors related to the patient and the disease carefully to minimize cancer-associated morbidity and mortality. The clinical use of cancer biomarkers is a valuable part of the clinical management of urological cancers. These biomarkers may lead to optimized detection, treatment, and follow-up of urological cancers. With the development of molecular research, newly developed biomarkers and next-generation sequencing have also contributed to patient management. In this chapter, we will present biomarkers in the most common urological cancers under subheadings of bladder cancer, prostate cancer, kidney cancer, and testicular cancer. Additionally, due to the development that occurred in the next-generation sequencing (NGS), all the above-mentioned malignancies are evaluated with regard to NGS.

Keywords: Biomarkers, Clinical management, Molecular research, Next-generation sequencing, Patient management, Urological cancers.

BIOMARKERS IN BLADDER CANCER

Bladder cancer (BC) is the 11th most commonly diagnosed cancer worldwide [1]. The most common symptom of BC is hematuria. Eighty-five percent of patients newly diagnosed with BC have painless macroscopic hematuria, and almost all present microscopic hematuria [2].

The gold standard test in the diagnosis of BC is cystoscopy. Currently, white light cystoscopy is the standard of care for the diagnosis of BC, and it is considered to be highly sensitive and specific for this type of cancer. However, it remains costly and somewhat invasive. As a result of the search for less invasive methods in the diagnosis and follow-up of bladder tumors, tissue-, blood- and urine-based biomarkers have also been developed.

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Pınar Atukeren and Hafize Uzun (Eds.)

Today, only urine-based biomarkers are used in clinical practice, and although many studies have been conducted on tissue- and blood-based biomarkers, none have found a place in clinical use. In this article, biomarkers are briefly discussed under separate sub-headings, and examples are given.

Blood-based Biomarkers

Tumor Cells

The presence of tumor cells in the blood has been associated with advanced-stage disease in solid organ malignancies. In a study investigating the prognostic effects of circulating tumor cells captured with the CellSerch system, the rates of recurrence, cancer-specific mortality, and overall survival (OS) were significantly lower in those with circulating tumor cells [3].

CD8 Count and Immune Cells

The CD8 count is a measure of the presence of cytotoxic T cells that may be involved in the immune response against tumor cells. One study reported that a low CD8 count was associated with recurrence-free survival after the transurethral resection of bladder tumor (TURT) [4]. However, the literature on the neutrophil-to-lymphocyte ratio (NLR), a marker of acute phase inflammation, is controversial. In a meta-analysis of 21 studies analyzing the prognostic role of NLR in BC, the authors correlated elevated pre-treatment NLR with OS, recurrence-free survival, and disease-free survival in BC [5]. In contrast, a secondary analysis of the SWOG 8710 trial reported that NLR was not a prognostic or predictive biomarker for OS in muscle-invasive bladder cancer, and it was not able to demonstrate OS benefit from neoadjuvant chemotherapy [6].

Matrix Metalloproteinase

Matrix metalloproteinases (MMPs) are calcium-dependent zinc-containing endopeptidases that are capable of degrading all types of extracellular matrix proteins. In the literature, several MMP isoforms have been simultaneously evaluated, but only MMP-7 levels have been associated with cancer-specific mortality [7].

Epigenetic Markers

Epigenetics investigate molecular DNA changes, including methylation and

histone alterations. These DNA changes are dynamic processes that determine which parts of DNA are more frequently transcribed and which regions are silenced. In one study, the promoter methylation of 17 genes was investigated, and that of five gene loci (RASSF1, E-cadherin, TNFSR25, EDNRB, and APC) was defined as a marker for tumor progression [8]. In another study, methylation at the RASSF1A and DAPK loci was identified as an independent predictor for tumor progression [9].

Tissue-based Biomarkers

Cell Cycle-based Markers

These biomarkers consist of p53, pRB, Ki67, cyclins, p21, and p27. Cell cycle regulators are among the most common mutated markers in BC and have been shown to be predictors of aggression, metastases, and cancer-specific mortality in many studies [10].

In a retrospective study with a large sample size, the authors examined four cell-cycle regulators (p53, pRB, p21, and p27) and found that a combination of the four markers was able to significantly improve the prediction of recurrence and cancer-specific mortality [11]. Prospective studies have also revealed that cell cycle-based markers can predict the upstage condition after TURT and the risk of recurrence after cystectomy [12, 13].

Transmembrane- and Signaling-based Markers

PDL/PDL1

In recent years, the programmed-death ligand (PDL) system has become an attractive alternative to treat urothelial carcinoma. PDL is a transmembrane protein pair that helps keep CD8 (cytotoxic) T cells from attacking normal cells. High levels of PD-L1 expression have been shown to be associated with advanced BC and poorer survival outcomes [14].

mTOR

The mammalian target of the rapamycin pathway regulates cell growth, proliferation, motility, and survival. These processes are dysregulated in the presence of multiple malignancies and generally downregulated in muscle-invasive bladder cancer [15].

CHAPTER 4

Circulating Biomarkers in Thyroid Cancer

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Abstract: Thyroid cancer is the most important endocrine cancer with increasing incidence. While thyroid cancers, especially papillary thyroid cancers, are known to exhibit generally a favorable outcome with excellent survival rates, some thyroid cancers are more aggressive with a poor prognosis. Several different biomarkers have been introduced for the diagnosis of disease, identification of tumor load, assessment of therapy response, and the detection of recurrence during follow-up of the thyroid cancer patients. This chapter gives a brief overview of the circulating biomarkers used in thyroid cancer patients.

Keywords: Biomarkers, Calcitonin, Carcinoembryonic antigen, Liquid biopsy, Thyroglobulin, Thyroid cancer.

INTRODUCTION

Thyroid cancer incidence has increased significantly over the last few decades, although the mortality rates have stayed relatively constant [1]. The demographics of thyroid cancer have also changed, with decreasing proportion of anaplastic thyroid cancers (ATC) and an increasing proportion of papillary thyroid cancers (PTC). Relatively stable mortality rates despite an increasing incidence have been attributed to the over-diagnosis of thyroid cancer as a result of better diagnostic tests, as well as to advancements in the treatment strategies of thyroid cancer.

Thyroid cancers are a heterogeneous group of tumors with cell types of different origin, developing from thyroid nodules and exhibiting different genetic alterations, biological behavior, and clinical course. Therefore, different biomarkers exist, each reflecting the unique biology of the selected tumor type. To date, there is not a single circulating biomarker that can be used to diagnose thyroid cancer has been found; rather, the diagnosis relies mainly on ultrasonography and fine-needle aspiration biopsy.

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Pınar Atukeren and Hafize Uzun (Eds.)

Thyroglobulin (Tg) is the most important biomarker for differentiated thyroid cancer (DTC), which originates from follicular epithelium, and calcitonin is the primary biomarker for medullary thyroid cancer (MTC), which stems from parafollicular cells of the thyroid gland. Basal serum calcitonin is a useful biomarker to determine the extent and presence of lymph node metastases in MTC patients.

Thyroid cancer is a malignant disease with generally a favorable prognosis, and relatively low rates of disease-related mortality compared to other cancers, and several studies have been conducted regarding biomarkers for diagnosis, therapy response assessment, and the follow-up of thyroid cancer patients. This chapter gives a brief overview on the histology of the thyroid gland, histopathology of thyroid cancers, their medical management and reviews the most important serum biomarkers that are used in the clinical management of thyroid cancer patients, as well as the liquid biopsy technique, which is a promising tool for the diagnosis and follow-up of thyroid cancer patients.

THYROID GLAND

The thyroid gland is located anterior to the larynx and consists of two lobes and a connecting isthmus in between. It is mainly composed of follicles, which consist of cuboidal-columnar epithelium filled with Tg-rich colloid. The major function of the thyroid follicles is to synthesize thyroid hormones, mainly thyroxine (T_4) and to a lesser extent triiodothyronine (T_3), both of which function in the regulation of metabolic rate and are required throughout human life. During fetal development and childhood, thyroid hormones are crucial for normal brain development, and they are required as regulators of intermediary metabolism in adults [2]. T_4 and T_3 are stored in great quantity in the extracellular colloid within Tg to avoid deleterious effects of thyroid hormone deficiency [2, 3].

Thyroid hormone synthesis and storage involve a unique process where iodide is necessary. Synthesis of thyroid hormones mainly comprises four stages: iodide trapping, which involves uptake of the circulating iodide by a membrane transport protein called sodium/iodide symporter (NIS) located in the basolateral membrane of follicular cells; synthesis of Tg within follicular cells; oxidation of iodide to form oxidized form of iodine by the enzyme thyroid peroxidase (TPO) located in the apical cell membrane and transport of iodine into the follicle cavity by pendrin; finally organification of Tg, which involves iodination of tyrosine residues of Tg within the colloid. Tyrosine is first iodized to monoiodotyrosine (MIT) and then to diiodotyrosine (DIT) by the enzyme TPO. The coupling of two DITs forms T_4, whereas the coupling of one MIT and one DIT forms T_3.

Thyroid follicle function is regulated by pituitary thyroid-stimulating hormone (TSH), also called thyrotropin, which stimulates both synthesis of thyroid hormones and their release into the systemic circulation. Upon TSH stimulation, the colloid is taken into the follicular cells and digested in lysosomes to liberate T_4 and T_3, and finally, T_4 and T_3 are released into the systemic circulation. MIT and DIT, which are freed from Tg during digestion, are not released into the blood; instead, they are recycled for new hormone synthesis.

Apart from thyroid follicles, the thyroid gland also has another cell type, namely parafollicular cells, or C cells, which are located as part of the follicular epithelium or as isolated clusters between thyroid follicles. Parafollicular cells are neuroendocrine cells that synthesize mainly calcitonin, which is a hormone responsible for calcium metabolism. When the serum calcium level is elevated, calcitonin is secreted, which in turn decreases bone resorption and increases calcium absorption by the skeletal system to normalize serum calcium levels. Carcinoembryonic antigen (CEA), adrenocorticotropic hormone (ACTH), B-melanocyte stimulating hormone, chromogranin, histaminase, neurotensin, and somatostatin are the other hormones or biological amines secreted by parafollicular cells.

CLASSIFICATION OF THYROID CANCER

Major subtypes of thyroid carcinomas are PTC (75-85%), follicular thyroid carcinoma (FTC) (10-20%), MTC (5%), and ATC (<5%) [4].

DTCs, including PTC and FTC, are derived from the follicular epithelium, and they preserve some of the normal thyrocyte functions, such as Tg synthesis and response to TSH stimulation. PTC is the most common thyroid cancer subtype in populations with sufficient iodine intake, whereas the incidence of FTC is higher in areas with iodine deficiency. Most patients with PTCs have favorable outcomes after appropriate disease management, whereas FTCs have a higher risk for recurrence and distant metastasis and lower survival rates compared to PTCs [5, 6]. Mutations in RET, RAS, or BRAF proto-oncogenes, all of which trigger the activation of mitogen-activated protein kinase (MAPK) cascade, are present in nearly 70% of PTC patients, and RAS mutated PTCs are typically follicular variant subtype of PTC [7 - 9]. RAS mutations and PAX8-PPARγ1 fusion are seen frequently in FTC patients [10, 11]. While cervical lymph nodes are the most common site of metastasis for PTC, hematogenous metastases to lungs and bones are more frequent in FTC [5].

MTCs are derived from parafollicular cells, therefore, unlike DTCs, they do not produce Tg; instead, they produce calcitonin, and they do not respond to TSH stimulation. Most MTCs are seen sporadically (70-80%), but familial autosomal

CHAPTER 5

DNA Methylation Biomarkers in Cancer: Current Clinical Utility and Future Perspectives

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Abstract: Epigenetic alterations are related to inherited but reversible changes in modifications that regulate gene activity beyond the DNA sequence. DNA methylation is the best characterized epigenetic modification, controlling DNA stability, DNA structure, transcription, and regulation, contributing to normal development and differentiation. In this section, we first discuss the cellular functions of DNA methylation and focus on how this fundamental biological process is impaired in cancer. Changes in DNA methylation status in cancer have been heralded as promising targets for the development of diagnostic, prognostic, and predictive biomarkers due to their noninvasive accessibility in bodily fluids (such as blood, urine, stool), reversibility, stability, and frequency. The absence of markers for definitive diagnosis of most types of cancer and, in some cases, DNA methylation biomarkers being more specific and sensitive than commonly used protein biomarkers indicate a strong need for continued research to expand DNA methylation markers. Although the information on changes in DNA methylation status in cancer and research on its clinical relevance is rapidly increasing, the number of DNA methylation biomarkers currently available as commercial tests is very small. Here, we focus on the importance of DNA methylation location and target genes likely to be developed in the future for the development of biomarkers in addition to existing commercial tests. Following a detailed study of possible target genes, we summarize the current clinical application status of the most studied and validated DNA methylation biomarkers, including *SEPT9, SDC2*, *BMP3, NDRG4, SFRP2, TFPI2, VIM* and *MGMT*.

Keywords: Biomarkers, Cancer, Carcinogenesis, CpG islands, DNA Damage, DNA Methylation, Genes, Gene Expression, Gene Silencing, Hypomethylation, Hypermethylation, Methyltransferases, Neoplasms, Oncogenes, Tumor, Tumor Suppressor.

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Pēnar Atukeren and Hafize Uzun (Eds.)

INTRODUCTION

Although cancer has been defined as a disease caused by mutations in driver genes for a long time, it is now accepted that epigenetic modifications also play a role in both the development and progression of the tumor [1]. Epigenetics is the field of science that studies gene activation or genetic changes in its function through processes, such as histone modification, DNA methylation, and acetylation without changing the DNA sequence. Although all cells in an organism contain the same genetic material, the changes that lead to the emergence of a wide variety of cell types are due to these epigenetic mechanisms [2]. In other words, epigenetic mechanisms are very important regulators for normal development and differentiation [3]. However, as can be predicted, the defects in this regulatory mechanism also lead to the emergence of various diseases such as cancer. If the cancer is to be mentioned, which has a complicated etiology, it should be noted that epigenome dysregulation, especially DNA methylation, is quite common in cancer and is associated with tumorigenesis and cancer phenotype [4]. Identifying epigenetic changes, such as DNA methylation and determining parameters that can be used as biomarkers, are extremely important, especially in determining the focal points of promising personalized treatment approaches. The main point of importance for these therapies is that, unlike genetic changes, such as mutations, epigenetic changes are inherited but reversible. In addition, DNA methylation markers are very important in terms of detecting diseases, such as cancer at an early stage.

DNA METHYLATION AND REGULATION MECHANISM

The best-known type of DNA methylation is defined as the formation of 5-methylcytosine (5mC) by attaching the methyl group ($-CH_3$) to the 5'-position of the pyrimidine ring of the cytosine (Fig. **1**). This methylation can occur in cytosines in the context of CpG, CHG, and CHH (where H refers to either A, C or T nucleotides), but in humans, it usually occurs in palindromic CG (or CpG) dinucleotides, and more rarely in non-CpG sequences [5, 6]. In recent years, it has been shown that N^6-methyladenine (N^6-mA) exists in the mammalian genome. Although it has been stated that this methylation may play a role in epigenetic regulation, its function has not been clearly clarified yet [7, 8]. 5-methylcytosine tends to spontaneously deaminate and undergo C-T transition. Because of this high mutagenicity, the distribution of CpG dinucleotides in the genome is thought to be unevenly distributed throughout the evolutionary process (also called CpG suppression) and is observed less frequently in the genome than expected. However, there are clusters of CpG sites, and such clusters are referred to as CpG islands (CGIs). CGIs are found in the promoter regions of 50% of the genes in the genome and are not methylated in normal cells, thereby transcription occurs.

Approximately 70-80% of the CpG sites are methylated; however, only 10% of the CpGs are methylated in the CGIs and active regulatory regions. These are CGIs in the promoter region of repressed genes such as X chromosome inactivated genes, imprinted genes and some tissue specific genes [6, 9 - 11]. Methylated cytosines do not occur randomly in the genome, they are largely found in transposable elements and repetitive DNA sequences, including centromeric and pericentric satellite DNA [12].

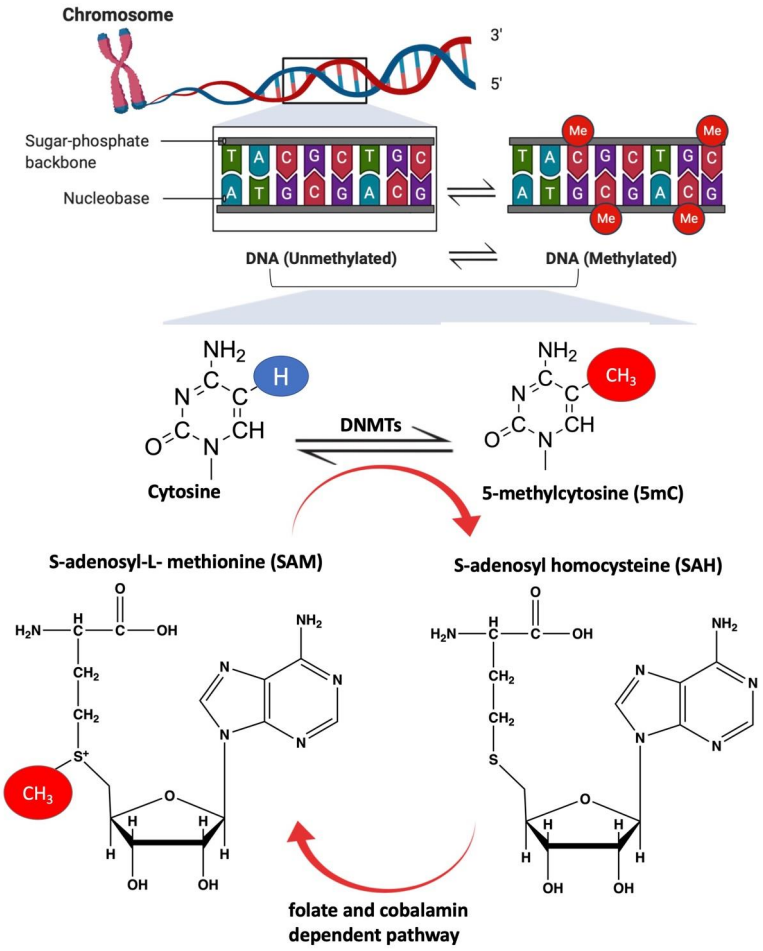

Fig. (1). DNA methylation. Formation of 5-methylcytosine (5mC) by attaching the methyl group from the methyl carrier, S-adenosyl-L- methionine (SAM), to the 5'-position of the pyrimidine ring of the cytosine.

DNA methylation is catalyzed by the DNA methyltransferase (DNMT) family that mediate the covalent addition of a methyl group from the methyl carrier, S-

CHAPTER 6

Circulating Biomarkers in Predicting Pathological Response to Neoadjuvant Therapy for Colorectal Cancer

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Abstract: Circulating biomarkers show promise in the management of many cancers. They have become the novel non-invasive approach to complement the current strategies in colorectal cancer (CRC) management. Their ability in guiding diagnosis, evaluating response to treatment, screening and prognosis is phenomenal, especially when it comes to their minimally invasive nature. These "liquid biopsies," which show potential for replacing invasive surgical biopsies, provide useful information on the primary and metastatic disease by providing an insight into cancer biology. Analysis of blood and body fluids for circulating tumour DNA (ctDNA), carcinoembryonic antigen (CEA), circulating tumour cells (CTC), or circulating micro RNA (miRNA) shows potential for improving CRC management. Recognizing a predictive model to assess response to neoadjuvant chemotherapy would help in better patient selection. This review was conducted with the aim of outlining the use of circulatory biomarkers in current practice and their effectiveness in the management of patients having CRC with a focus on response to neoadjuvant therapy.

Keywords: Biomarkers, Colon cancer, Neoadjuvant therapy.

INTRODUCTION

Colorectal cancer (CRC) is the second commonest cancer diagnosed in females (approximately 820,000 cases in 2018) and the third commonest cancer in males (approximately 1,020,000 cases in 2018) [1]. It is the second most commonest cause of cancer-related death globally, with approximately 900,000 deaths occurring annually [1]. There is a rising incidence of CRC in both developing and developed countries [2, 3]. The pathogenesis includes a series of genetic and epigenetic mutations causing alterations in cell growth, differentiation, and apoptosis, transforming the normal colonic epithelium into an adenocarcinoma. Loss of oncosuppressor genes, such as *APC*, *TP53,* and a series of molecular alte-

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Pınar Atukeren and Hafize Uzun (Eds.)

rations in proto-oncogenes, such as *KRAS,* lead to progression of an adenomatous polyp to invasive carcinoma in a stepwise fashion, which is known as the adenoma-carcinoma sequence [4].

The prognosis of CRC largely depends on the stage of the disease at diagnosis, and therefore, early detection is known to improve morbidity and mortality. Conventional means of disease assessment such as radiological imaging and histopathological analysis have minimum sensitivity in terms of early detection of systemic spread. Therefore, in recent years, the concept of liquid biopsy, which includes the analysis of circulatory biomarkers in blood and body fluids, has emerged as a promising diagnostic tool [5]. The diagnosis and management of CRC have been generally based on biopsy-based techniques for decades. However, determining the individualised response to treatment and the prognosis is often difficult based solely on the histological assessment [6]. This has led to the discovery of biomarkers for early detection of relapse and treatment resistance leading to the improved overall quality of life and patient survival [7]. There is ample evidence to suggest that biomarkers such as CTC in peripheral blood are associated with a negative impact on patient survival [8]. In contrast to the conventional tissue biopsies, liquid biopsies are associated with considerable benefits such as minimal invasiveness, less pain and lower procedural risks. The term "liquid biopsy" reflects a much broader perspective which refers to the utility of all circulating tumour traits, including CTC, ctDNA, circulating miRNA, exosomes, proteins, and mRNAs in the peripheral blood and body fluids [9]. An ideal biomarker should have properties including the ability to quantify, high specificity, reliability, measurability, predictability, and sensitivity [10].

Neoadjuvant chemotherapy revolutionised the management of CRC by substantially reducing the risk of local recurrence and improving outcomes, especially in locally advanced cancers. However, the response to neoadjuvant chemotherapy varies ranging from pathological complete response (pCR) to no response. Pre-operative chemotherapy has its own associated disadvantages, including significant adverse effects which may interfere with timely surgery. The use of circulatory biomarkers in predicting response to neoadjuvant therapy would help select patients who are more likely to respond to such therapy.

CRC restaging after neoadjuvant chemotherapy is equally important, as it will determine the presence of nodal metastasis, involvement of the circumferential resection margin, and the depth of invasion in rectal carcinoma [11]. Conventional imaging modalities, namely computed tomography (CT), magnetic resonance imaging (MRI), endoscopic ultrasonography (EUS), and positron emission tomography (PET) are used in the process of restaging. However, the overall accuracy of these investigations is reduced in restaging after neoadjuvant therapy

in comparison to the initial staging. This is mainly due to failure of differentiating tumour infiltration and residual tumour from radiation-induced fibrosis. Radiation related complications, including proctitis, may also be misinterpreted as local invasion [11, 12].

Among patients receiving neoadjuvant CRT for rectal carcinoma, a significant proportion (15-40%) has been shown to achieve a pCR. Such patients tend to have an excellent prognosis with an almost non-existent risk of local recurrence and more than 95% five-year overall survival [13, 14]. There is an ongoing debate regarding the possibility of managing these patients without surgery, sparing the postoperative and long-term morbidity associated with radical surgical resections [15]. However, classification as pCR mandates a histological examination of the entire specimen which is not possible without surgical resection. Therefore, a complete clinical response (cCR) which is the absence of clinically detectable tumour after neoadjuvant CRT is used as a surrogate for pCR. Assessing for cCR would depend on clinical examination findings correlated with endoscopy radiological investigations and may also include histological examination of biopsies from the previous tumour site [16]. Surgery is usually timed within 10 to 12 weeks following completion of CRT, which allows adequate time for tumour regression. Management of patients who have achieved cCR to neoadjuvant therapy still remains a controversial topic, where non-operative management is becoming a popular alternative in this group, especially among patients who are at high risk for surgery. Although controversial, a combination of frequent clinical, endoscopic and radiographic evaluation have been shown to be helpful in ensuring local tumour control and detecting early recurrences [17]. Patients after completion of 10-12 weeks of neoadjuvant CRT with evidence of residual tumour, should undergo surgery. The rest with a cCR may be given the option for surgical resection or watch and wait strategy. Those who opt for non-operative management should be monitored closely for 1-year after neoadjuvant CRT followed by a long-term follow up protocol [13, 18]. As cCR is only a surrogate of pCR, the utility of circulatory biomarkers in this scenario is a matter of great interest.

The objective of this comprehensive review is to outline the use of circulatory biomarkers in current practice and their effectiveness in the management of patients with colorectal cancer. This review summarises tissue-based biomarkers in blood, blood-based biomarkers, circulating peptides, and other novel biomarkers in CRC with a special emphasis on predicting response to neoadjuvant therapy in CRC.

CHAPTER 7

Circulating Biomarkers in the Management of Breast Cancer

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Abstract: Circulating biomarkers have become a promising modality in the management of many cancers. Similarly, in breast cancer, circulatory biomarkers are useful, non-invasive methods in the diagnosis, prognostication, and evaluation of response to treatment. Invasive surgical biopsies can be potentially replaced by "liquid biopsy," which involves analysing circulatory biomarkers that may reveal features of primary and metastatic disease. Therefore, providing an insight into the cancer biology can be utilised to monitor treatment response, treatment-induced adaptation and tumour and disease progression through non-invasive means. The objective of this review is to provide an overview of the current status of the circulating biomarkers highlighting their promising impact on the management of patients with breast cancer.

Keywords: Biomarkers, Breast cancer, Circulatory biomarkers, Diagnosis, Prognostication, Liquid biopsy.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer worldwide, being a leading cause of cancer death among women in both developing and developed countries [1, 2]. However, the survival rates are improving due to the advances in screening, diagnosis, understanding of tumour biology and targeted systemic therapy [3]. Furthermore, the role of surgery and invasive approaches in the management of breast cancer has evolved substantially over the past century, where radical approaches have gradually been replaced by more conservative approaches [4]. The triple assessment is used as the 'gold standard' in the assessment of symptomatic breast lumps, which comprises a clinical examination, radiological investigations and histopathological analyses [5]. Circulating biomarkers are promising non-invasive surrogates for tissue diagnosis. They are defined as soluble molecules released to the bloodstream by tumour cells or cells in the

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Pınar Atukeren and Hafize Uzun (Eds.)

tumour microenvironment or response to the tumour [6]. Analysis of these biomarkers would provide useful insight into the molecular makeup of cancer.

The study of circulatory biomarkers in the form of circulating tumour cells (CTC) dates back to the late 1800s although, they were studied extensively after the mid-20th century. Their presence was generally considered to be associated with a poorer prognosis [7]. Similar to other solid malignancies, breast cancer is associated with changes in immune [8], glycoproteomic [9], proteomics [10], and nucleic acid [11] biomarkers in the blood, which arise directly from the tumour cells. A measure of these indicators can be acquired by simply drawing peripheral blood, which is less invasive and more feasible compared with tissue biopsy. With the advancements of molecular diagnostics, this method of "liquid biopsy" for biomarkers helps immensely in clinical management [12].

Although many potential biomarkers have been identified, only a few are used in clinical practice which include carcinoembryonic antigen (CEA), MUC-1 protein, human epidermal growth factor receptor-2 (HER-2), and cytokeratins [7]. Recently the interest has shifted towards other markers, including cell-free plasma DNA (cfDNA), circulating tumour cells (CTCs) and microRNAs (miRNAs) [13]. However, there is still an ongoing debate concerning their usefulness in patient monitoring, follow-up and assessment of response to therapy. These biomarkers lack reliability when considered singularly due to their intrinsic lack of specificity and sensitivity. However, certain biomarkers such as circulating tumour cells likely will have a predictive value in specific situations including diagnosis of metastatic carcinoma [14]. With the advancing interest in these markers, it is evident that they provide valuable information on the status of malignancy both qualitatively and quantitatively [14]. These biomarkers are useful in analysing the molecular characteristics of cancer cells in instances where tissue samples cannot be obtained or are not available and as a valuable tool in monitoring the therapeutic response of the tumour and to assess residual disease after treatment [13]. The purpose of this review is to objectively summarize the current recommendations on different types of circulating biomarkers in breast cancer, their usefulness in the diagnosis, prognosis, and monitoring of response to treatment.

ESTABLISHED BIOMARKERS

Carcinoembryonic Antigen (CEA)

CEA is a serum-based glycosylated oncofoetal glycoprotein expressed in normal mucosal cells and overexpressed by adenocarcinomas of the colon, rectum, breast, pancreas, and lungs [15, 16]. CEA is useful in the monitoring metastatic breast cancer during treatment [15, 16]. Immunobiological studies have revealed that the

domain structure of CEA proteins belonging to a large family of glycoproteins closely resembles the gamma heavy chain of the immunoglobulin IgG [6]. Being a member of the immunoglobulin superfamily, CEA attaches on to cell membranes by a glycosyl phosphatidylinositol (GPI) anchor [17]. The exact mechanism on how it is released to the extracellular matrix still remains unclear. However, experiments have revealed that similar to other GPI anchored proteins, CEA could be released through a GPI anchor cleavage catalysis, mediated by an endogenous enzyme called glycosylphosphatidylinositol-specific phospholipase D [18].

Although the exact function of CEA has not been identified yet, recent studies portray an involvement in cancer growth, invasion and metastasis by playing a role in cell migration [19]. Elevated CEA levels are observed in approximately 50-60% of patients with metastatic breast cancer [20]. However, this marker can only be accurately interpreted in combination with normal levels of CA15-3 and CA 27.29 [20]. Increased levels of CEA and CA 15-3 have been shown to be independent prognostic factors of metastatic breast cancer. CA 15-3 elevation is more marked in younger patients while CEA is a good prognostic marker in the context of older patients with estrogen receptor (ER) negative disease. The two biomarkers were also shown to provide information on recurrence and overall survival in metastatic breast cancer [21].

Elevated levels of CEA in the preoperative context generally devise a poorer prognosis when interpreted in combination with CA 15-3 [22]. Ebeling at al studied a sample of 1046 women on the association of CEA and CA 15-3 with disease free survival and death from breast cancer [23]. In that study, the postoperative decrease of CEA was an independent prognostic factor for both these study outcomes. Furthermore, multivariate comparison of preoperative and post-operative CEA levels showed a decrease in postoperative levels by more than 33% to be associated with a higher risk of relapse and death [23]. This finding was further confirmed by a similar study which also showed that raised preoperative values of CEA was associated with overall unfavourable cancer status with regards to tumour burden, lymph-node metastasis and tumour stage leading to poorer clinical outcomes [21]. Furthermore, this study also showed that reduced CEA and CA 15-3 levels predicted an overall better outcome [21]. It is quite noteworthy that the reliability and the prognostic values improved when the two biomarkers are used in combination rather than in singular where both the sensitivity and the specificity dropped substantially [21]. Although these classic biomarkers were found to be of considerable clinical importance, they may not be objectively representative of a reliable panel in the context of a liquid biopsy.

CHAPTER 8

Clinical Application of Biomarkers for Hematologic Malignancies

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Abstract: Over the last decade, significant advancements have been made in the molecular mechanisms, diagnostic methods, prognostication, and treatment options in hematologic malignancies. As the treatment landscape continues to expand, personalized treatment is much more important.

With the development of new technologies, more sensitive evaluation of residual disease using flow cytometry and next generation sequencing is possible nowadays. Although some conventional biomarkers preserve their significance, novel potential biomarkers accurately detect the mutational landscape of different cancers, and also, serve as prognostic and predictive biomarkers, which can be used in evaluating therapy responses and relapses. It is likely that we will be able to offer a more targeted and risk-adapted therapeutic approach to patients with hematologic malignancies guided by these potential biomarkers. This chapter summarizes the biomarkers used (or proposed to be used) in the diagnosis and/or monitoring of hematologic neoplasms.

Keywords: Chromosomal, Classification, Cytogenetic, Epigenetic, Genomic biomarkers, Hematologic neoplasm, Immunohistochemistry, lncRNA, Microenvironment, MicroRNA, Minimal residual disease, Molecular, Mutations, Scoring system.

INTRODUCTION

There are many markers (both traditional and novel) described for hematologic malignancies that might play a role in the pathogenesis, diagnosis, and prognosis. These biomarkers may also act as targets that might have the potential for providing new therapeutic approaches.

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Pınar Atukeren and Hafize Uzun (Eds.)

Hematologic malignancies can be categorized as lymphoid and myeloid. The most recent World Health Organization (WHO) classification of these malignancies includes many subgroups [1, 2]. In this chapter, we try to summarize the biomarkers used (or proposed to be used) in the diagnosis and/or monitoring of these neoplasms.

BIOMARKERS IN MYELOID NEOPLASMS

This part of the chapter mainly focuses on the biomarkers for the myeloid neoplasms such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasms (MPNs), including chronic myeloid leukemia (CML).

Acute Myeloid Leukemia

AML arises from the clonal expansion of malignant hematopoietic precursor cells in the bone marrow. AML is the second most common leukemia in adults and the most common type of acute leukemia. The median age at diagnosis is approximately 65 years, and the incidence increases with age. There is a slight predominance in men and a higher incidence in non-Hispanic whites than other racial and ethnic groups.

AML has been associated with environmental factors like exposure to chemicals, radiation, tobacco, chemotherapy, and retroviruses. In some cases, AML precedes other clonal hematopoietic disorders such as MDS, MPNs, paroxysmal nocturnal hemoglobinuria, and aplastic anemia.

Initial genetic analyses are not practical in AML because of the genetic heterogeneity of the disease. This makes the diagnosis difficult and as a result, influences therapeutic strategy negatively [3]. AML is lethal if untreated, and with intensive treatment, 60-80% of younger adults achieve a complete remission (CR), and almost one-third are finally cured. However, a 5-year relative survival rate of AML is approximately 25%. Outcomes are heterogenous and overall survival (OS) rates range between 5-70% [4, 5]. Therefore, prognostic markers to guide therapy and predict outcome are still needed. Prognostic markers can be clinical, disease-related, and molecular. However, the strongest prognostic factor for predicting therapeutic response and survival is cytogenetics.

Multiparameter flow cytometry (MFC) immunophenotyping gives information for AML diagnosis, classification, and monitoring. MFC allows identification, quantification, and lineage assessment of blasts and disease classification according to the maturation stage. AML confers very heterogeneous immunophenotypic features, mainly due to the genetic diversity of the disease [3].

Performing precise risk stratification due to immunophenotypic profiles can be difficult. There are not any exact recommendations and/or any reliable approaches for the use of new biomarkers in the immunophenotypic panels for AML as well as for the markers that might have an effect on prognosis and survival in clinical practice [3].

CYTOGENETIC CLASSIFICATION OF AML

The WHO criteria for the diagnosis and classification of AML are based on morphologic, immunophenotypic, cytogenetic, and molecular features [2, 6]. This categorization provides information for prognosis and may be useful for the selection of the best therapy [7]. The WHO classification has replaced the former French-American-British (FAB) classification system, which mainly depended on morphology and cytogenetic features [2, 8] (Tables **1** and **2**).

Table 1. WHO classification of AML and related malignancies (adapted from reference [2]).

AML with Recurrent Genetic Abnormalities	AML, NOS
AML with t(8;21) (q22;q22.1); *RUNX1-RUNX1T1*	AML with minimal differantiation
AML with inv(16) (p13.1q22) or t(16;16) (p13.1;q22); *CBFB-MYH11*	AML without maturation
APL with *PML-RARA*	AML with maturation
AML with t(9;11) (p21.3;q23.3); *MLLT3-KMT2A*	Acute myelomonocytic leukemia
AML with t(6;9) (p23;q34.1); *DEK-NUP214*	Acute monoblastic/monocytic leukemia
AML with inv(3) (q21.3q26.2) or t(3;3) (q21.3;q26.2); *GATA2, MECOM*	Pure erythroid leukemia
AML (megakaryoblastic) with t(1;22) (p13.3;q13.3); *RBM15-MKL1*	Acute megakaryoblastic leukemia
AML with *BCR-ABL1*	Acute basophilic leukemia
AML with mutated *NPM1*	Acute panmyelosis with myelofibrosis
AML with biallelic mutations of *CEBPA*	Myeloid sarcoma
AML with mutated *RUNX1*	Myeloid proliferations related to Down syndrome
AML with myelodysplasia-related changes	Transient abnormal myelopoiesis (TAM)
Therapy-related myeloid neoplasms	Myeloid leukemia associated with Down syndrome

CHAPTER 9

Applications of Biomarkers in Cancer Surgery

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Abstract: The most problematic and difficult cases of general surgery applications are cancers. Cancer can be diagnosed in many ways, including the presence of some symptoms and signs, screening tests, and imaging methods. The diagnosis of cancer is confirmed anatomically by microscopic examination of tissue samples. Biomarker research has become increasingly interesting for many medical fields today. Biomarkers are indicators of the biological process that can show changes in disease and health status and reveal pathological conditions. The sensitivity of markers used for early and painless diagnosis is weak in all types of cancer, including thyroid, breast, stomach, pancreatic, and colorectal cancers. Biochemical and molecular markers have been found to be of great importance in cancer research in recent years in the formation mechanisms and treatment steps of cancer. Several biomarkers have been studied in the circulating body fluids and tissue of preoperative, intraoperative, and postoperative patients. The main use of tumor markers is the follow-up of the response to treatment and early detection of recurrences in patients diagnosed with cancer. Tumor markers correctly selected will guide the patient's monitoring and treatment planning. But, still, the field of cancer surgery needs new biomarkers.

Keywords: Biomarkers, Breast cancers, Colorectal cancers, Pancreatic cancers, Stomach cancers, Surgery, Thyroid cancers.

INTRODUCTION

Biomarkers are molecules that can evaluate biological and pathological processes and are present in measurable amounts in blood, body fluids, or tissues. Biomarkers used in surgery may play a role in early diagnosis, risk stratification, evaluation, and metastasis and prognosis prediction [1]. An ideal biomarker should be determined easily in body fluids as plasma and urine samples, predict relevant clinical outcomes and reflect prognosis and treatment efficacy. These evaluations are essential (essential/indispensable/ crucial) to establish an appropriate treatment and recruit the patient. The properties sought in an ideal biomarker are as follows: it is biochemically stable, it can be studied with low

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Pınar Atukeren and Hafize Uzun (Eds.)

blood amounts or body fluids, its sampling time is wide, it can be detected with a fast, cheap, simple, and automated system, its sensitivity is 100%, and its specificity is> 85%, it can distinguish bacterial and viral pathogens, and it is associated with inflammatory response [2].

Clinical use of circulating tumor markers include neoplasia screening, risk identification, cancer diagnosis, clinical classification of cancer, treatment selection, monitoring response to treatment, determination of the prognosis, recurrence detection, and metastasis tracking. Tumor markers are rather used after the initial treatment is completed to define the disease state and to evaluate the treatment options. Detection of cancer recurrence allows re-initiation of treatment or changes in treatment [3].

Although high levels of tumor markers are seen in malignancies, many hormonal and metabolic changes can be encountered in benign diseases. Sometimes a tumor may not produce the substance it is associated with until the advanced stage. A substance may not be specific to a single tumor type. In other words, these items are not sensitive and specific enough. However, they are useful in monitoring a malignancy with a tissue diagnosis, treatment selection, and survival prediction [4 - 6]. Tumor markers are often used in combination with immunohistochemical tests to diagnose cancer.

The most known and used tumor markers are shown in Tables **1**, **2** and **3**.

Table 1. The most known and used enzyme tumor markers in routine clinical use.

ENZYME TUMOR MARKERS			
-	**Malignancy**	**Non-neoplastic Causes**	**Clinical Utility**
Prostate specific antigen (PSA)	Prostate cancer	Prostatitis, prostate hypertrophy	Screening, therapy monitoring, and recurrence
Lactate dehydrogenase (LDH)	Hematologic malignancies, lymphoma, Ewing sarcoma, Germ cell	Hepatitis, hemolytic anemia	Prognostic indicator and elevated nonspecifically in numerous cancers.
Alkaline phosphatase (ALP)	Metastatic carcinoma of bone, hepatocellular carcinoma, osteosarcoma, lymphoma, leukemia.	Growth-development, pregnancy, bone diseases, liver diseases	Determination of liver and bone involvement; nonspecific elevation in many bone-related and liver cancers.
Neuron-specific enolase (NSE)	Neuroendocrine tumors, Small cell lung cancer, neuroblastoma	Septic shock, pneumonia, and nervous system trauma	Prognostic indicator and monitoring disease progression for neuroendocrine tumors.

Table 2. The most known and used endocrine tumor markers in routine clinical use.

h-CG	**Gestational trophoblastic diseases, germ cell tumors**	**Pregnancy, renal failure, heterophile antibody**	**Diagnosis of choriocarcinoma, testicular cancer**
Adrenocorticotropic hormone (ACTH)	Pituitary adenoma, ectopic ACTH-producing tumor	Cushing syndrome	Diagnosis of ectopic ACTH-producing tumor
Calcitonin	MTC and neuroendocrine tumors	Chronic renal failure, Zollinger-Ellison syndrome, pernicious anemia	Screening, response to therapy, and monitoring recurrence of MTC
Growth hormone (GH)	Pituitary adenoma, ectopic GH-secreting tumors	Acromegaly, dwarfism, gigantism	Diagnosis and post monitoring of acromegaly
Prolactin	Pituitary adenoma	Irregular or no periods, infertility, breast milk discharge in non-pregnant, tenderness in the breast	Diagnosis and postsurgical monitoring of prolactinoma
Antidiuretic hormone (ADH)	Posterior pituitary tumors	Guillain-Barré syndrome, multiple sclerosis, epilepsy, and acute intermittent porphyria	Diagnosis of SIADH
Cortisol	Adrenal tumors	Cushing syndrome, excess cortisol, adrenal insufficiency or Addison disease, deficient cortisol.	Diagnosis of Cushing's syndrome, adrenal adenoma

Table 3. The most known and used tumor markers as oncofetal proteins in routine clinical. use.

α- fetoprotein (AFP)	**Hepatocellular carcinoma, Hepatoblastoma, Non-semitomatous germ cell tumours**	**Hepatitis, cirrhosis, biliary obstruction,**	**Hepatocellular carcinoma (screening, diagnosis, treatment monitoring), hepatoblastoma (diagnosis), non-semitomatous germ cell tumours (prognosis, treatment monitoring)**
Carcinoembryonic antigen (CEA)	Colorectal carcinoma (main), Other gastrointestinal malignancies, breast and ovarian carcinoma.	Cigarette smoking, bronchitis, hepatitis, alcoholic liver disease, ulcerative colitis, Crohn's disease and pancreatitis.	Prognosis, treatment monitoring and recurrence of colorectal carcinoma.

CHAPTER 10

A Pathophysiological Approach To Current Biomarkers

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Abstract: Biomarkers are necessary for screening and diagnosing numerous diseases, predicting the prognosis of patients, and following-up treatment and the course of the patient. Everyday new biomarkers are being used in clinics for these purposes. This section will discuss the physiological roles of the various current biomarkers in a healthy person and the pathophysiological mechanisms underlying the release of these biomarkers. This chapter aims to gain a new perspective for evaluating and interpreting the most current biomarkers.

Keywords: AFP, Alpha-fetoprotein, CA15-3, CA27.29, Carcinoembryonic antigen, CEA, GFAP, GPBB, GSTP-1, H-FABP, MUC1, NSE, NT-proBNP, Pathophysiological mechanisms, Physiology, S100B, Troponin.

CARDIAC BIOMARKERS

Cardiac biomarkers are widely used to detect functional heart problems and predict possible heart disease that may clinically show up later. The most common use of cardiac biomarkers is for acute myocardial infarction and congestive heart diseases, but they can also be used for a wide variety of diseases [1].

Cardiac biomarkers are, in general, intracellular components of myocytes or enzymes, formally. This title will discuss the pathophysiological mechanisms of typical and recent cardiac biomarkers, and the intracellular pathways of the release of these biomarkers will be explained in detail.

Cardiac Troponins

Troponin functions as a Ca^{+2} receptor and regulates Ca^{+2} levels during myosin and actin's interaction for contraction in skeletal and cardiac muscles. It is shown that

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Pınar Atukeren and Hafize Uzun (Eds.)

troponin spreads through the thin filaments of a muscle with a length of 38 nm. Troponin that runs along the thin filament and attaches to active sites of tropomyosin settles on the grooves of actin. When intracellular Ca^{+2} levels decrease, troponin suppresses the contractile interaction between actin and myosin with the help of tropomyosin. On the contrary, this suppression is removed when intracellular Ca^{+2} levels increase as Ca^{+2} ions bind to troponin [2].

Troponin complex consists of three different subunits: troponin I, C, and T. They all have different functions such as removing of interaction between actin and myosin (troponin I), binding to Ca^{+2} (troponin C), and binding to tropomyosin (troponin T). Also, studies have shown that several mutations in genes that encode cardiac troponin subtypes are associated with inherited cardiomyopathies [2].

Cardiac troponin subtypes are widely used for myocardial ischemia. Before the discovery of troponin, different biomarkers were used to identify myocardial ischemia, such as aspartate transaminase (AST), lactate dehydrogenase (LDH), and creatinine kinase (CK). These biomarkers are still in use, but none show specificity for cardiac muscle. The later found biomarkers showed more specificity to cardiac muscle, such as CK-MB and LDH, but they still had a high false positivity rate. Troponins were first found in 1965, but the routine laboratory usage as a marker started in the 1990s. Troponin measurements have up to 100% sensitivity when observed 6 – 12 hours after the start of chest pain, and they also have high specificity for damaged cardiac muscle. Because of these benefits, high troponin levels were added as a criterion of myocardial infarction in "Third Universal Definition of Myocardial Infarction", which is the current definition used by the American College of Cardiology [3 - 5].

Troponin Subunits

Troponin consists of three different subunits: troponin I, C, and T. The primary function of the troponin is inhibiting the interaction between actin and myosin, which is directly related to intracellular Ca^{+2} levels.

Troponin I: Troponin I directly inhibits the contractile interaction between myosin and actin. This inhibition occurs with the absence of Ca+2 and is also very strong in the presence of tropomyosin. A After discovering troponin I, studies with synthetic troponins showed a region composed of residues 105–114 (minimum inhibitory peptide), called the 'inhibitory region.' It is relieved that this region is essential for showing the inhibitory activity with the help of a secondary region of residues 140–148 (second actin-binding region). The presence of this secondary region is also necessary for total inhibition. The N-terminus of troponin I, which is close to the inhibitory site, attaches to troponin C and together with troponin T, they all build a helix-coil structure.

On the other hand, the C-terminus attaches to the tropomyosin and actin complex. The N-terminal region that binds to troponin C- Ca^{+2} dependent troponin – is called the 'switch region.' This switch region is constantly phosphorylated and dephosphorylated *via* protein kinase A (PKA). When PKA phosphorylates the switch region, the affinity of troponin C with Ca^{+2} decreases, which inhibits the contraction of muscle as well. This mechanism is essential for the sympathetic nervous system to decrease diastole duration with increasing intracellular cyclic adenosine monophosphate (cAMP) levels and cAMP-related PKA activity *via* β_2 receptors [6].

Troponin C: Troponin C is the main subunit that reacts with Ca and forms a complex structure with troponin I and troponin T. The primary function of troponin C is suppressing the inhibition of the interaction between actin and myosin formed mainly by tropomyosin. Troponin C can bind calcium *via* four active sites located in the N terminus. Sites 1 and 2 are formally low-affinity Ca+2 receptors, while sites 3 and 4 are both high-affinity Ca^{+2} and Mg^{+2} receptors. This explains the necessity of magnesium in striated muscle contraction as well as in cardiac contraction. The binding of Ca^{+2} to these sites induces a conformational change resulting from a hydrophobic site that connects to troponin I to open (open conformation). After binding to troponin I, they form a helical structure and inhibit the suppression of troponin I-troponin T-tropomyosin complex on actin [6].

Troponin T: Troponin T is the subunit that binds to tropomyosin and also attracts other troponins. Troponin T is a tropomyosin-binding subunit of troponin. Troponin T is the largest of the three troponins and interacts with both troponins I and troponin C. Troponin T consists of two subunits: TnT1 and TnT2, which bind to N-terminus and C-terminus of tropomyosin, respectively. The role of troponin T is not directly regulating contractile interaction, but it works as an indirect factor. Troponin T adjusts the sensitivity of troponin C to Ca^{+2} with its subunit TnT2. The N-terminal part of cardiac troponin T (cTnT) contains 30 more residues than troponin T in striated muscle. These residues contain mainly exons, and by alternative splicing of these exons, four different isoforms can be produced in the human heart, and all these variants show different sensitivity to Ca^{+2}. Also, aberrant splicing of these exons due to genetic and epigenetic changes may alter the ventricular pumping function [7].

Regulatory Function of Troponin

The primary function of the troponin complex is to regulate Ca^{+2} with two related mechanisms: inhibiting actin-tropomyosin complex *via* troponin T and interacting between troponin C and troponin I, depending on intracellular Ca^{+2} levels. The

CHAPTER 11

Biomarkers in Otorhinolaryngology

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Abstract: Biomarkers of otorhinolaryngologic diseases with higher insult over a person's him/herself and overall health services are summarized in brief. In order to define, diagnose, treat and monitor any disease markers are needed. Otorhinolaryngology (ORL) is interested in special disease entities of the region besides otorhinolaryngologic involvements of the systemic diseases and unique forms of pathologies such as cholesteatoma, Meniere's disease and otosclerosis. Neoplasia is another heading to deal with. In the following chapter, one will find an overview of molecules that have been used as a biomarker as well as the end points of the present research on the issue relevant with ORL. Day by day, new molecules are being named however, the pathways of action are rather the same. Readers will find the headings related to the most common diseases of the field, informing them about where to look for defining new strategies of understanding of each disease.

Keywords: Biomarkers, laryngology, Biomarkers, otology, Biomarkers, rhinology, Biomarkers, thyroid.

INTRODUCTION

Biomarkers are used to diagnose, define the pathophysiology, determine therapeutic alternatives and monitor the outcome by means of both curative attempts and natural history of the disease, which may overall be named as prognosis. In the present chapter, biomarkers of otorhinolaryngologic diseases that have more insult over a person's him/herself and overall health services are summarized in brief. Protein can be monitored *via* their code/decode patterns in the course of a specific disease, namely fragmented RNA particles, mRNA, or DNA's. Owing to the higher cost of the genetic laboratory, it seems more feasible to define biochemical reflections of protein synthesis. It is not rational to list any diseases, but rather frequent diseases are tried to be given with their future-lightening markers by means of diagnosis, disease monitoring and prognosis.

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Pınar Atukeren and Hafize Uzun (Eds.)

Rhinology

In rhinology practice, infections, namely sinusitis acute of chronic, space occupying benign masses of sino-nasal tract polys or papilloma, are frequent. Main underlying pathology emerges as allergy. Immune reactions of any type can be monitored *via* re-popularized hematological parameters such as PMN/Lymphocyte ratio, Platelet number or platelet volume, however most of those are far from being specific for an illness. They can be monitored as an adjunctive measure but cannot discriminate a confounding simultaneous inflammatory process from the primary disease of concern.

Sinusitis is basically divided into two as acute or chronic. While acute sinusitis is an infection caused by mainly bacteria, chronic sinusitis is a complex disease entity related to various inflammatory pathways. CRS is classified into CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). CRS, especially CRSwNP, is further subdivided into eosinophilic CRS (ECRS) and non-eosinophilic CRS. According to the diagnostic criteria established by a multicenter study called the Japan Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis Study (JESREC Study) in 2015, JESREC score and mucosal eosinophil count have been used to diagnose eosinophilic chronic rhinosinusitis (ECRS) [1].

TH2 inflammation was formerly reported to predominate which is associated with elevated levels of eosinophils and cytokines, including Interleukin (IL)-4, IL-5, and IL-13 [2].

The testing mediums to obtain CRS biomarkers are peripheral blood, nasal secretions and nasal lavage, tissue biopsies from the sinuses, and nasally exhaled breath. CRSwNP is an eosinophilic process and CRSsNP is noneosinophilic. In different parts of the world, eosinophilia may be absent in the pathogenesis of CRSwNP, yet if present is generally associated with higher disease severity. Additionally, eosinophilic polyps are generally more glucocorticoid responsive in comparison to non-eosinophilic counterparts.

IgE, as an inducer of eosinophilia, is utilized as a biomarker for CRS phenotyping. Patients with CRS frequently express high levels of IgE in both serum and local secretions. Allergic sensitization to bacterial antigens is believed to be an important part of the process. Staphylococcus aureus enterotoxins can serve to activate basophil degranulation in those with CRSwNP. Increased local IgE is predictive of recurrence requiring repetitive surgical intervention. The omalizumab, an anti-IgE therapeutic, was also tested and provided benefit up to some extent in patients with CRSwNP. Cytokine profile of CRS w/s NP differs dramatically. In CRSsNP, neutrophilia dominates with the expression of

Transforming Growth Factor β (TGF-β), type I interferons, and IL-6, IL-8, or IL-17. CRSwNP has a TH2 leading microenvironment with marked expression of thymic stromal lymphoprotein (TSLP) and type 2 inflammatory cells. Type 2 Innate Lymphoid (ILC-2) cells, which produce IL-4, IL-5, IL-13, IL-25, and/or IL-33, activate T and B cells that overall result in increased leaking of the epithelial barrier. Evidence nonetheless points to elevated IL-4 playing a role in the overexpression of cysteinyl leukotriene [13]. CD45+ CD4- IL-4 producing cells were found to be elevated in patients with AERD (asprin exacerbating resp disease) [3].

There can be an overlap of cytokine expressions of both TH 1 and 2 pathways. The presence of IL-5 may be defined for a specific endotype of CRSwNP individuals with more severe disease. IL-25 expression is upregulated in CRSwNP tissue is associated with increased severity in CT scores. Anti IL-4,5,13, 25 are used in several trials and provided unneglectable outcomes. Eotaxin-3 as a plasma biomarker for mucosal eosinophil infiltration is closely related to IL-33 and TSLP levels which indicate respiratory diseases [4]. Eotaxin-3 and total IgE in the nasal secretions of patients receiving dupilumab, a monoclonal IgG4 antibody against the alpha subunit of the IL-4 receptor, decreased in comparison to the placebo group, while eosinophilic cathionic protein (ECP) did not decrease significantly. Tissue analysis of nasal polyp revealed that while total IgE, ECP, eotaxin-2, eotaxin-3, IL-13 and PARC are significantly lowered in the dupilumab group; IL-6, IL-1β, IL-4, eotaxin-1, IL-5, IL-10, IL-17, IL-33, TNF-α or TARC are not significantly different.

Longitudinal tracking of biomarkers is important to objectively measure therapeutic efficacy. Cytokines have local and indirect systemic effects of long term. IL 4 and IL-13 has a direct effect on Periostin, that is an extracellular protein taking place in subepithelial fibrosis in airway tract through interacting with integrins. and working with VEGF, resulting in angiogenesis. It was shown that Periostin levels are treatment responsive as the protein works with tissue remodeling factors. IL-16 is an eosinophilic chemotaxis that promotes persistent eosinophil activation with increased levels of serum IL-16 is significantly elevated in eosinophilic CRS [5].

An ATP-dependent transmembrane efflux pump, P-glycoprotein is upregulated in TH2 disease that is thought to take place in CRS-related inflammation through promotion of cytokine secretion. Verapamil is an antagonist of p- glycoprotein, and preliminary trials show that low dose verapamil therapy is safe and effective for CRSwNP treatment [6]. Autoimmunity seems to play a greater role. CXCL-12 and CXCL-13 molecules are present with increased levels, which enhance B-cell chemotaxis. Anti-dsDNA IgG antibody is correlated with a worse clinic. The

CHAPTER 12

Pharmacogenomic Biomarkers

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Abstract: Why does the usual dose of medication work for a person while another individual cannot give the expected response to the same drug? On the other hand, how come half of the usual dose of an analgesic relieves an individual's pain immediately, as another man continue to suffer even after taking double dose? Although a treatment method has been successfully used in majority of the population for many years, why does the same therapy cause serious side effects in another region of the world? Most presently approved therapies are not effective in all patients. For example, 20-40% of patients with depression respond poorly or not at all to antidepressant drug therapy. Many patients are resistant to the effects of antiasthmatics and antiulcer drugs or drug treatment of hyperlipidemia and many other diseases. The reason for all those is basically interindividual differences in genomic structures of people, which are explained in this chapter in terms of the systems and the most frequently used drugs in clinical treatment.

Keywords: Adverse reactions, Carriers, Clinical pharmacology, Cytochrome P-450 enzyme family, Drug response, Enzyme activity, Extensive metabolizer, Intermediate metabolizer, Metabolism, Optimal dosage, Personalized medicine, Pharmacodynamics, Pharmacogenetics, Pharmacogenomic biomarkers, Pharmacokinetics, Polymorphism, Poor metabolizer, Targeted treatment, Transporter molecules, Ultra-rapid metabolizer.

INTRODUCTION

Personalized medicine has been an outstanding subject for overcoming this challenge and providing each individual with a tailored treatment rather than the traditional method of "trial-and-error" dosing. The primary goals of personalized medicine are to minimize the potential adverse drug reactions and obtain the optimal efficacy from the medication.

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Pınar Atukeren and Hafize Uzun (Eds.)

Many drugs were withdrawn from the market because they caused severe adverse effects, so targeted drugadministration or dosing based on an individual's genetics has become important to avoid withdrawals. Moreover, the optimum efficacy can be reached when the drug is administered in the right dosage for each individual. For instance, 40-50% of patients with asthma, migraine, or diabetes cannot benefit from their medications, while the response rate of patients to cancer chemotherapy is only 25%.

The individual risk for drug inefficacy or drug toxicity is a product of the interaction between genes and the environment. The environmental variables can be nutritional factors, lifestyle influences such as smoking, barbequed meat or alcohol consumption, and also diseases or concomitantly administered drugs [1]. These factors act in concert with an individual's genes that code for pharmacokinetic and pharmacodynamic determinants of a drug, such as receptors, ion channels, drug-metabolizing enzymes, and drug transporters. In brief, the aim of personalized medicine is to give the right drug in the right dose at the right time to the right patient, having the right symptom for optimum efficacy while avoiding adverse effects [2].

Pharmacogenetics deals with the effect of individual gene variants on drug action. It investigates the simple relation between hereditary factors and the diversity of drug response. Simple mutations, which play important roles in the response of an individual to a specific drug, are determined by combining genetics and pharmacological methods. On the other hand, genomics involves the systematic identification of all human genes and gene products. It is the study of human genetic variations in health and disease, as well as changes in gene and protein expression over time. Pharmacogenetics has been evolved into pharmacogenomics to study the entire genomic structure that determines drug response, including the diversity of the human genome sequence and its clinical consequences. According to the definition of Food and Drug Administration (FDA), "Pharmacogenomics is the discipline for the estimation of drug response of an individual based on the genetic structure." Genetic information is being used to guide both drug and dosage selection for individual patients in clinical practice.

Pharmacogenomic biomarkers are variations, leading to person-to-person differences in the metabolism of drugs and thus, in drug kinetics and response. Single nucleotide polymorphisms (SNPs) are single-base differences that exist between individuals with a frequency of more than 1%, and they are the most common types of variations among pharmacogenomic biomarkers. An SNP may cause an amino acid substitution during transcription (non-synonymous/ missense), which affects protein activity; on the other hand, it may not result in the amino acid change (synonymous), but still, it may alter the gene expression

and the amount of the product. Other types of variations include insertion, deletion, inversion of the gene, encoding the protein of interest, and also copy number variation, tandem duplication, dispersed duplication, mobile element insertion, or translocation (Fig. **1**).

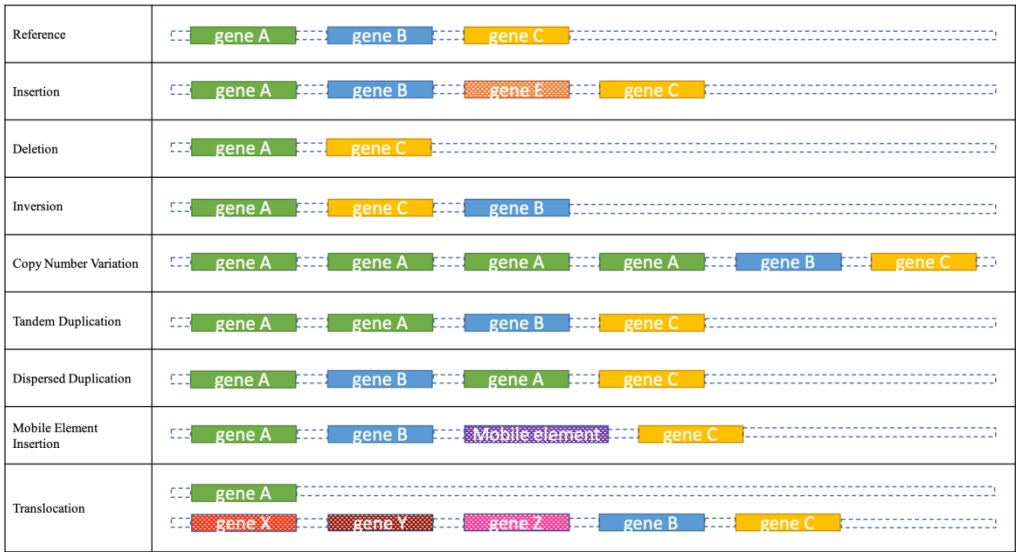

Fig. (1). Different types of genetic variations.

Polymorphisms and other genetic variations commonly occur for genes encoding proteins that function in drug metabolism, drug transporters, and drug target proteins. Drug metabolism and drug transporter genotypes can alter drug availability at the target site. In addition, drug target genotypes can affect a patient's sensitivity to a drug. If a large number of variations and their frequencies in different populations are known, they can be used to generate an individual's genetic fingerprint, indicating the individual's potential drug response to any given drug. Thus, those biomarkers can be used to provide a drug response profile, which is the resulting phenotype after all of the known affecting variations are contributed.

There are two main concepts, which are pharmacokinetics and pharmacodynamics, investigating the relationship between a drug and the human body after administration; there are specific proteins working in the process. If there is one of any aforementioned variations on the encoding gene of a protein, its function may be changed, which constitutes differences in the individual's drug response profile.

CHAPTER 13

Clinical Application of Circulating MicroRNAs as Novel Biomarkers for Different Diseases

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Abstract: Biomarker research has become increasingly interesting in many areas nowadays. Biomarkers are indicators of the biological process that can show changes in disease and health status and reveal pathological conditions. There is always a need for markers that divide patients into risk categories that can help in early diagnosis, detect complications ahead of time, guide treatment, and predict adverse outcomes in a chronic complex and certain diseases such as cancer. microRNAs (miRNAs) are ~ 22 nt long npcRNAs involved in post-transcriptional arrangements. miRNAs regulate messenger RNAs (mRNA), especially through negative regulation of gene expression. The fact that miRNAs have come to the fore in many disease mechanisms brings up their use as biomarkers in the early stage. The purpose of this review is to gather the latest information on this subject by bringing together recent articles and reviews to contribute to understanding the role of miRNAs, which act as biomarkers in different ways in vital processes, in the formation, early diagnosis, and treatment of diseases. miRNAs have an important potential to become a next-generation biomarker and therapeutic. But, each miRNA molecule can bind to a large number of different mRNAs, and different miRNAs in each mRNA. Therefore, new findings are needed to determine the expression activities and targets of miRNAs.

Keywords: Autoimmune Diseases, Biomarkers, Cancer, Coronary Artery Disease, Genital Diseases, Female, MicroRNAs, Nervous System Diseases, SARS-CoV-2.

INTRODUCTION

A feature that can be objectively measured and evaluated, which can be an indicator of pharmacological response to normal biological processes, patho-

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logical processes, or a therapeutic process, is called a "biomarker." This definition was developed in 2001 by the "Biomarkers Definitions Working Group" [1].

Biomarkers are biological signals originating from the human body. In this case, virtually any biological sign, if measurable, *e.g.*, biological material analysis, electrocardiography (ECG), blood pressure, etc., are all biomarkers.

The Purpose of Biomarkers

1. Diagnosis

2. Disease staging

3. Risk identification

4. Disease prognosis

5. Treatment evaluation

6. Treatment follow-up

7. Population screening

An ideal biomarker [1] should be biochemically stable and able to be studied in a small blood volume [2]; sampling time should be wide (> 48 hours); It should be detected by fast, cheap, simple, and automated analysis method [3]; results should be comparable between laboratories, and the threshold value should be well established [4]; sensitivity/negative predictive value should be 100%, and specificity/positive predictive value> 85% [5]; should be able to predict a complicated course in determining prognosis, and [6] should have features that can demonstrate the efficacy of treatment.

MicroRNAs (miRNAs)

miRNAs are a class of endogenous small RNAs and are functional RNA molecules that are transcribed from the intron or exon regions encoding protein on the genome and RNA genes in regions that do not encode a protein and are not translated into protein [2]. miRNAs consist of an average of 20-22 nucleotides. They regulate post-transcriptional gene expression by targeting messenger RNA (mRNA). Suppression of translation or fragmentation of the target mRNA with the relationship between miRNA and mRNA are possible consequences that may occur. But what if miRNAs are more than gene regulation? Many studies have shown that the miRNA profile in the blood of patients changes compared to

healthy people. These discoveries have been of great interest in the use of the level of extracellular (extracellular) miRNAs as a novel biomarker in various diseases [3, 4].

miRNAs have the ability to leave the cell. Extracellular miRNAs were first discovered in a cell culture medium. Extracellular miRNAs encapsulated in exosomes and secreted from donor cells could be transferred to other recipient cells. The presence of extracellular miRNAs in human body fluids has been mentioned in several studies. Extracellular miRNAs have been discovered in various macromolecules in blood and other body fluids. As a result of disease or other necrotic events, cells die, and miRNAs are released passively from the cytoplasm, which can be detected in the blood. Exosomal miRNAs from miRNAs released outside the cell are transported in the vesicle covered with a lipid bilayer membrane. These are more stable than free circulating miRNAs [5].

A biomarker based on miRNA levels has several advantages over biomarkers currently used at the levels of specific proteins in the blood [5, 6].

1. The strongest advantage is that the global miRNA level can be measured quickly and accurately.

2. Because of the high precision and efficiency of transcriptome profiling techniques, it is much easier to profile global miRNA levels than proteins or metabolites.

3. The miRNA population consisting of different sequences in humans can be rapidly measured with high-throughput sequencing technology because many miRNAs are expressed differently in different tissues and cellular situations. It can help more with the combination of miRNA levels.

4. The origin of cancer tissues can be classified at the miRNA level.

5. If we compare it with protein or other types of molecules where no amplification method can be applied, polymerase chain reaction (PCR) based amplification enables measurement of the low amount of miRNA in body fluids, even in small volumes.

The fact that miRNAs have come to the fore in many disease mechanisms brings to the agenda their use as biomarkers in the early stage. Much research has been done to find extracellular miRNAs that can be used as diagnostic or prognostic markers for specific diseases. After the first promising results of the studies, it turned out that the exact quantification of circulating miRNAs is more complex than expected. One of the uncertainties it brings is why the expression profiles of

CHAPTER 14

Advancements in Gastrointestinal System Biomarkers

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Abstract: The requirement for diagnostic surgical operations of gastrointestinal system diseases significantly decreases with the help of proper diagnostic tools. These modalities are also beneficial for identifying postoperative complications, allowing us to diagnose them in earlier stages, and increasing the postoperative survival rates. Biomarkers are considered an integral part of diagnostic examinations. Developments of biomarkers used for diagnosing and treating abdominal diseases are essential for improving our capabilities in non-invasive monitoring. In this chapter, we review both the novel and the routine biomarkers in the diagnosis and follow-up of gastrointestinal system diseases.

Keywords: Benign Gastrointestinal System Diseases, Biomarker, Diagnostic biomarkers, Malignant Gastrointestinal System Diseases, Surgery.

INTRODUCTION

In the last century, great advancements have been made in the preoperative preparation, intraoperative technique, postoperative follow-up, and treatment stages in surgery. While these advancements facilitate our routine surgical procedures, they also increase disease-free survival rates after surgery. Especially in the last thirty years, surgery has enormously evolved in an attempt to ensure minimally invasive operations.

With the advancements in surgery, the routine practices we use in the screening and diagnosis of diseases have also advanced. These advances reached their pinnacle in biochemical and radiological examinations. Thus, differential diagnosis of acute abdominal diseases can be performed much easily, and the

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need for diagnostic laparotomy has decreased significantly. Brilliant advances in endoscopic methods have paved the way for minimally invasive interventions, especially in the screening, diagnosis, and treatment of gastrointestinal diseases.

These advancements allowed surgeons to perform cost-effective, easily accessible procedures which cause the least trauma for the patient. Although previous studies have focused on the structure and functions of these biomarkers, recent studies have also focused on their clinical usage. Therefore, the clinical usage of biochemical markers as diagnostic tests and patient follow-ups has gradually increased. This chapter focuses on biomarkers that we are now using or incorporating into our clinical practice, which do not require excessive surgical procedures or post-operative pathological evaluation.

Diagnostic Biomarkers of Upper Gastrointestinal System Diseases

Upper gastrointestinal system diseases are one of the most common causes of global hospital admissions. More than 10 million applications for upper gastrointestinal system complaints were made in the United States in 2018 [1]. According to 2019 clinical data, out of 1,806,590 cancer cases in the United States, 333,680 were diagnosed in the gastrointestinal system and approximately 46,000 in the upper gastrointestinal tract (stomach and esophagus) [2]. Many methods have been developed for the diagnosis and treatment of these disorders, and undoubtedly endoscopic interventions and radiological examinations account for a large percentage. According to the clinical data of 2013, 6.1 million upper gastrointestinal system endoscopies were performed in the United States [3]. However, the fact that endoscopic interventions are considered invasive, which may also require anesthesia for the procedure, and their occasional inappropriate usage, researchers are looking for easier methods for diagnosing these disorders.

Diagnostic Biomarkers of Benign Upper Gastrointestinal System Diseases

Pepsinogen I & II

Pepsinogen (PG) is the zymogenic form of pepsin and it is classified as an endoproteinase with two different isoforms. While Pepsinogen I is released from the chief cells and neck cells in the stomach fundus and corpus, Pepsinogen II is secreted from Brunner glands in cardia, antrum, pylorus, and duodenum [1, 4]. It turns into its activated form pepsin in acidic conditions and takes part in the hydrolysis of nutritional proteins. Pepsinogens I & II are useful markers that can reveal gastritis. Especially in *Helicobacter pylori*-associated gastritis, both PG values elevate in serum, predominantly PG II [5, 6]. A rapid decrease in PG II

after the treatment of H.pylori infection makes serum PGs useful in the control after eradication treatment [5]. Furthermore, PG tests can be used as a confirmation test for other non-invasive tests, as a high false positivity rate can be seen in other non-invasive tests such as urea breath test and *H. pylori* stool antigen test, especially in acute cases with a low prevalence of *H. pylori* [6].

PG values can also be used to diagnose atrophic gastritis. In chronic atrophic gastritis cases in which the corpus is affected, a decrease in PG values is observed. It has been reported that serum PG I - PG II values and PG I / II ratio may be useful for the early diagnosis of gastric atrophy [7, 8].

The use of serum PGs in gastric cancer screening is also increasing day by day. Recent studies have shown that there is a possible link between low serum PG I value and the risk of stomach cancer due to atrophic gastritis [9, 10]. In the study conducted in asymptomatic patient groups, higher rates of gastric cancer and intestinal-type intramucosal cancer were observed in upper GIS endoscopy in patients with low PG values (PG test positive) compared to the patients with normal PG values (PG test negative) [11]. Thus, it is thought that serum PG values may be a useful biomarker in terms of screening for gastric cancer in the patient population with high risk.

Gastrin 17

Gastrin 17 (G-17) is a peptide hormone consisting of 17 amino acids produced by antral G cells, and it provides the control of gastric acid secretion with the help of a negative feedback mechanism. G-17 release is directly related to intragastric acidity, so it is a significant indicator of gastric acidity [12].

Gastroesophageal reflux (GERD) and reflux-related diseases are conditions in which gastric acidity increases significantly. Thus, G-17 values are found to be decreased in GERD and reflux-related diseases. A study in which patients with retrosternal burning complaints were evaluated showed a correlation between different reflux patterns and G - 17 values, and no additional invasive procedures were required [13]. Barrett's esophagitis (BE) is another reflux-related disorder that occurs due to high gastric acidity. BE patients have lower G - 17 levels compared to normal patients, suggesting that it may help the diagnosis for early detection of esophageal cancer [12].

Another condition which both gastric acidity and G - 17 affected is atrophic gastritis. The stomach can be evaluated in two different segments from the corpus-antrum junction; the proximal segment mainly produces exocrine secretion, and the distal segment is widely responsible for the endocrine secretion

CHAPTER 15

Novel Applications of Biomarkers in Chronic Obstructive Pulmonary Disease

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Abstract: Chronic obstructive pulmonary disease (COPD) is an important health problem and an increasing cause of morbidity and mortality worldwide. Currently, COPD is considered a multisystem disease. Although it primarily affects the lungs, structural and functional changes occur in other organs due to systemic inflammation. It is stated that in patients with COPD, airway and systemic inflammatory markers are increased and that these markers are high are associated with a faster decline in lung functions. In recent years, numerous articles have been published on the discovery and evaluation of biomarkers in COPD. Many markers have also been studied to accurately assess COPD exacerbations and provide effective treatment. However, based on the evidence from published studies, a single molecule has not been adequately validated for broad clinical use.

Keywords: Chronic obstructive pulmonary disease, Biomarkers, Exacerbation, Therapy.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) was previously defined only by the presence of airflow limitation, is now defined as an inflammatory disease. It is also stated that COPD is a systemic disease, and comorbid and common comorbidities contribute to the severity and mortality of COPD. In COPD, the musculoskeletal system (muscle dysfunction and osteoporosis), cardiovascular system (atherosclerosis), endocrine, and nervous system are mainly affected [1]. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), interleukin-8 (IL-8), IL-6, tumor necrosis factor-alpha (TNF-α), and leukotriene B4 (LTB4) are the major inflammatory markers that may cause COPD. The importance of changes in these markers during exacerbation and after treatment has been emphasized in various studies [2].

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Pınar Atukeren and Hafize Uzun (Eds.)

With the demonstration that many inflammatory cells, cytokines and enzymes are involved in different stages of COPD, the place and importance of inflammation in the pathophysiology of the disease has been better understood in recent years. Many inflammatory cells and markers are involved in the pathogenesis of COPD and this inflammation has been demonstrated by different methods. Developments in this area will also help develop new approaches to the treatment of the disease [3].

Biomarkers are measurable indicator molecules that show biological developments, pathological changes, and responses to pharmacological treatment. A good biomarker should be disease-related, sensitive, reliable, consistent, reproducible, practically applicable, and should also be generalizable and cost-effective; these can be cells, cytokines, proteins, genes, and products, *etc* [4].

To show inflammation in COPD and other respiratory system diseases, samples of bronchial biopsy, induced sputum, bronchoalveolar lavage (BAL) fluid, and expiratory air can be taken. Many inflammatory markers can be measured in BAL fluid. Eosinophil cationic protein (ECP), myeloperoxidase and IL-8 levels were found to be higher in COPD and smokers than in healthy non-smokers. Protease and antiproteases can be detected in BAL fluid. In COPD, there is an increase in total elastase activity and a decrease in anti-elastase activity, which reveals the protease-antiprotease imbalance compared to normal smokers. It has been reported that the ratio of neutrophils and macrophages is increased in BAL fluid and sputum in patients with COPD. Neutrophils increase the release of proteases, such as neutrophil elastase and matrix metalloproteinase (MMP), and toxic peptides, such as oxidants and defensins in COPD. While neutrophil ratios in bronchial biopsies and induced sputum correlate with the severity of the disease, they also show parallelism with the rate of decline in pulmonary function tests. Macrophages are significantly increased in the airways, lung parenchyma, BAL fluid, and sputum. Macrophages increase the release of TNF-α and chemokines sensitive to some monocytes, especially with the effect of smoking in COPD. Macrophages also cause the release of many MMPs such as MMP-1, MMP-9, and MMP-12. In stable COPD patients, activated T-lymphocytes such as macrophages and especially CD-8 T-lymphocytes, interferon (IFN) -γ, CXCL10 (IFN---induced protein [IP-10]), IL-4 and IL- 9 oscillate. CD-8 T-cells cause a variety of inflammatory changes. CD-8 T-cells have been demonstrated to increase the airways of patients with COPD, and it is known that the CD-4/CD-8 ratio reverses in COPD. Natural killer (NK) cells increase numerically at a low rate in terms of phagocytosis ability in patients with COPD. However, more pronounced increases in NK cell counts were found in the sputum of COPD patients, especially during acute attack periods [5 - 8].

Increases in the number of neutrophils, CD-8 T cells, and sometimes eosinophils are observed in the induced sputum of patients with COPD. The increase in eosinophils helps identify coexisting asthma or higher bronchodilator response and subgroups of patients who will benefit from corticosteroids [3, 6, 9]. Increased levels of IL-8 in the sputum of patients with COPD correlate with bacterial load in the airways and myeloperoxidase released from activated neutrophils. IL-6, IL-1β, TNF-α, and IL-8 were increased in induced sputum; monocyte chemotactic factor-1 (MCF-1) increases, causing IL-8 release in the bronchial epithelium. Cytokines are proteins that are generally smaller than 80 kilodaltons and enable communication between cells. Cytokines have important roles in inflammation, and they function with surface receptors with high affinity for them. TNF-α increases ICAM-1 level in patients with COPD. The activation of macrophages results in the formation of MMP. In bronchial epithelial cells, extracellular TNF-α plays an important role in the production of tenascin, a matrix glycoprotein. Increased serum TNF-α concentration has been shown to be associated with weight loss in COPD. TNF-α and receptors in COPD are higher than non-smokers. IL-8 is a neutrophil-specific chemotactic factor and a CXC chemokine that activates LTB4 and neutrophil 5-lipoxygenase. LTB4 is known to provide chemotactic activity in the sputum of patients with COPD. IL-8 also functions as a chemotactic for T-cells. IL-8 was found to be higher in induced sputum in COPD patients compared to smokers without COPD and the control group. The value of IL-8 increases even more with the severity of the decrease in FEV1 values, and this becomes more pronounced in attacks. Leptin, another important cytokine, is detected in the induced sputum of patients with COPD and correlated with TNF-α and other inflammatory markers such as CRP [10].

Measurement of markers in exhaled air is preferred due to its noninvasiveness and easy reproducibility. Nitric oxide (NO) in the expiratory air (eNO, FeNO) has been extensively studied in asthma. However, the clinical benefit of measuring eNO in COPD is limited and normal or slight elevations can be seen. The increase in eNO in COPD correlates with the increase in bronchodilator and corticosteroid response, just like the increase in eosinophils [11, 12]. It is not useful for practical use as it is highly variable due to environmental CO levels and the effects of passive smoking Concentrated exhaled breath condensate (EBC) is easy to make It has been preferred frequently in recent years due to its nature and non-invasiveness, protein biomarkers in exhaled air could not be measured reliably by conventional methods [13, 14]. Hydrogen peroxide (H_2O_2) and 8-isoprostane, which are among oxidative stress markers, are found to be high in the EBCs of patients with COPD. pH is lower. While pro-inflammatory cytokines, including IL-1, IL-6, and TNF-α, are detected higher in exhalation air during COPD exacerbation, some important markers are fully observed in EBCs [6, 14].

CHAPTER 16

Biomarkers in Diabetes Mellitus

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Abstract: Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia that occurs as a result of impaired insulin secretion and/or insulin effect, or both of these factors. The fact that the disease has both individual and social dimensions makes it important to detect as early as possible and make the necessary lifestyle changes. For this purpose, it becomes necessary to develop fast, effortless, cheap, and reliable methods for diagnosis. We discussed which biochemical markers should enter routine use according to their sensitivity and specificity among the biochemical markers that have been used and are still being studied. In this chapter, we explored some methods that may be used as biomarkers and discussed advantages and pitfalls for each.

Keywords: Advanced glycation end-products, Biomarkers, Diabetes mellitus, Glycated albumin, HbA1c, microRNAs, Netrin, Salivary glucose.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease presents with hyperglycemia caused by impaired insulin secretion or insulin resistance and/or destruction of pancreatic β cells. By 2019, IDF declared that approximately 463 million adults were suffering from DM, and by 2045, this number is projected as 700 M. DM also caused 4.2 M deaths in the aforementioned year [1].

DM is classified into three major subgroups; Type I (5-10%), Type II (most common, 90-95%), gestational DM, and some other specific types. Type I diabetes mostly occurring in juveniles is immune-mediated and insulin-dependent diabetes. Patients with Type I DM have to use subcutaneous insulin injections several times a day to survive. Type II diabetes, also known as insulin-independent diabetes, is a result of impaired cellular insulin response, which may be called insulin resistance. In patients with type II DM, there is also a phase called pre-diabetes, which presents insulin resistance and impaired fasting plasma

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Pınar Atukeren and Hafize Uzun (Eds.)

glucose (FPG) levels. Insulin resistance and Type II DM are associated with obesity, age, and family history [1].

With novel approaches in DM treatment, it is possible to prevent DM progression before causing microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (coronary artery disease, peripheral arterial disease, and stroke) complications. In this context, detecting DM at the pre-diabetes phase provides better control of this disease. Thus, new diagnostic techniques and tools will help us to detect DM as earlier as possible so we can start treatment in an early phase of diabetes.

Currently, FPG and hemoglobin A1c (glycated hemoglobin, HbA1c) measurements are in use as a biomarker at DM diagnosis. There is also an oral glucose tolerance test (OGTT) for detecting pre-diabetes and gestational diabetes. DM is diagnosed with;

- An FPG level of 126 mg/dL (7.0 mmol/L) or higher, *or*
- A 2-hour plasma glucose level of 200 mg/dL (11.1 mmol/L) or higher during a 75-g OGTT, *or*
- Random plasma glucose of 200 mg/dL (11.1 mmol/L) or higher in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, *or*
- An HbA1c level of 6.5% (48 mmol/mol) or higher [2].

HbA1c is currently a reference test being used for diagnosis, screening, and follow-up in patients with DM. HbA1c reflects blood glucose levels over a 2-3 months period and is insufficient with rapid glycemic changes. Furthermore, it is not recommended in patients with uremia, hemoglobinopathy, anemia, and pregnancy. Patients with those situations are very common in daily clinical practice. Thus, alternative biomarkers would help clinicians for better diagnosis and follow-up in patients with DM.

In this chapter, we will explore some other methods may be used as biomarkers.

GLYCATED ALBUMIN (GA)

Albumin is the most abundant protein in plasma. With a molecular weight of 66.7 kDa, it contains a single polypeptide chain with 585 amino acids and 17 disulfide chains. Albumin makes up approximately 60% of total serum protein with concentrations in a range of 30-50 g/L and a half-life of 14 to 20 days [3].

Glycation is defined as a non-enzymatic spontaneous reaction that occurs in situations with high plasma glucose, initiated with the addition of reducing sugars and/or their metabolites to amine groups (typically lysine and arginine) within

proteins. With forming a Schiff base, an intermediate product can undergo a rearrangement to create a more stable Amadori product. After dehydration and oxidation, reactive dicarbonyl compounds transform in to precursors of advanced glycation end-products (AGEs). This incident is known as the Maillard reaction [3].

Albumin glycation is affected by glucose concentration and the time of exposure between glucose and protein [4]. Compared to hemoglobin, albumin glycation rates are about 9 to 10 times greater [5]. Ueda and Matsumoto conducted an *in vitro* experiment, and they showed that GA production was 4.5 times greater than HbA1c production after adding the same glucose concentrations. That showed GA production is faster in the same *in vitro* conditions [6].

There are several ways to measure GA like ion-exchange high-performance liquid chromatography (HPLC), boronate affinity chromatography, immunoassays, and colorimetric method with thiobarbituric acid and enzymatic methods using proteinase and ketamine oxidase [7]. Enzymatic methods result in a shorter time and are easy to perform compared to other methods [8]. In all methods, GA levels increase 2 to 5 fold in patients with DM compared to healthy individuals [3].

GA is being considered as a biomarker for DM diagnosis since Tomigana and colleagues conducted a study to determine a reference interval for GA in 2006, which resulted in a range of 12.3% to 16.9% in 699 patients [8]. In 2011, a study with a larger patient number (N=1575) reported a similar interval with a cut-off point for DM diagnosis as 15.5% shows 83.3% sensitivity and specificity by using fasting glucose (≥126 mg/dL) and Hba1c (≥6.5%) as reference tests [9]. In years following, studies showed similar cut-off points with similar sensitivity and specificity rates.

After diagnosis, GA has also been considered as a consistent follow-up biomarker. As mentioned before, GA has a half-life of 14-20 days, making it suitable for detecting rapid glucose changes in a shorter time than Hba1c with a mean life of 120 days. GA can show the efficacy of drug therapy [10] and insulin treatment with a better correlation with fasting glucose than HbA1c [11]. Thus, GA is more useful for managing the medication or dosage of oral antidiabetics and titration of insulin therapy.

Long-term complications of DM are another challenge to predict by biomarkers. A study showed GA and HbA1c had a similar association with microvascular complications like retinopathy and nephropathy. Same study showed cardiovascular complications were predicted by only HbA1c [12].

CHAPTER 17

Biomarkers and their Clinical Applications in Pediatrics

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Abstract: Biomarker studies are becoming increasingly interesting for many fields of medicine. The use of biomarkers in medicine is involved in detecting diseases and supporting diagnosis and treatment decisions. New research and new discoveries on the molecular basis of the disease show that there may be a number of promising new biomarkers for use in daily clinical practice. Clinical trials in children lag behind adult research both in quality and quantity. The number of biomarkers validated to optimize pediatric patient management is limited. In the pathogenesis of many diseases, it should not be extrapolated to the pediatric clinical setting, taking into account that biomarkers that are effective in adults are clearly different in children and that ontogeny directly affects disease development and therapeutic response in children. The search for ideal biomarkers or markers that can make an early and definitive diagnosis in neonatal sepsis is still ongoing. The ideal biomarker for pediatric diseases should be cost-effective, noninvasive, applicable to pediatric specific diseases, and its results should correspond to age-related physiological changes. Lactate, troponin and B-type natriuretic peptide are valuable biomarkers in the evaluation and management of critically ill children with cardiac disease. Tumor markers in children are biochemical substances used in the clinical treatment of pediatric tumors and to detect the presence of cancer (regression or progression). In this chapter, current and brief information about biomarkers and their clinical applications used in the diagnosis and monitoring of pediatric diseases is presented.

Keywords: Biomarker, Early predictor, Management, Monitoring, Premature, Tumor markers.

INTRODUCTION

Biomarker research has become increasingly interesting for many areas of medicine today. Biomarkers (biological markers) are indicators of the biological process that can show changes in disease and health status and pathological conditions. Biomarkers may include biofluids (urine, blood, saliva, stool, amniotic

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Pınar Atukeren and Hafize Uzun (Eds.)

fluid, bronchoalveolar lavage, cerebral spinal fluid), other tissue (including skin, hair or nails), radiographic, genetic, or any other physiologic testing. While biomarker provides important information in the diagnosis of a disease, they may be ineffective in showing the course of the disease. However, even if a biomarker may not be a good diagnostic biomarker, there are numerous other types of potential applications for newly discovered markers. For example, prostate-specific antigen (PSA) used in prostate cancer is an important parameter for diagnosis, but it is not a suitable marker for monitoring the disease. Similarly, carcinoembryonic antigen (CEA) used in ovarian carcinomas is not an important indicator for diagnosis, but it is a good biomarker in the follow-up of the disease [1, 2]. The most important characteristics of an ideal biomarker are summarized in Table **1**.

Table 1. The most important characteristics of an ideal biomarker.

• It should be specific for the tumor type identified.
• Analysis should not require an expensive tool.
• The results of the biomarker should be easily interpreted.
• It must have analytical and clinical validity.
• Its quantity should not show a wide variation in the general population.
• It must be precise, sensitive and specific for the condition of the disease.
• Its measurement is practical and easy; its levels should be compatible with the stages of the disease.
• Adequate and reliable amounts of body tissues and fluids should be obtained.

Despite the lack of knowledge on child-specific biomarkers, their use is beneficial as it is integrated into the pediatrician's daily practice. When a complete blood count (leukocyte) and C-reactive protein (CRP) are measured for the initial evaluation of bacterial infection in a febrile child or HbA1c to assess glycemic control in a diabetic patient, biological surrogates are used to assess pediatric disease processes and direct the care plan. There are many approaches when considering pediatric biomarkers. A more traditional approach uses well-established adult biomarkers and applies data directly to children. This approach is similar to the drug development process where a drug is developed for adult disease, studied in adult patients, and then investigated for potential use in children [3]. This chapter reviews and summarizes the different types of biomarkers and their clinical applications in many childhood diseases. Although many diseases have overlapping biological system involvement, they were broadly categorized as noncommunicable diseases, metabolic diseases, inflammatory diseases, liver diseases, urological diseases, autoimmune diseases, genetic diseases, and rare diseases.

CHILDHOOD NONCOMMUNICABLE DISEASES

Noncommunicable diseases (NCDs), such as cardiovascular diseases, cancer, diabetes and chronic respiratory diseases, are the leading global cause of death and are responsible for just over 70% of deaths worldwide [4].

Biomarkers in Patients with NCDs in Childhood

Allergy, Asthma, Atopic Dermatitis (AD)

Allergy is not one disease but a collection of a number of allergic conditions. The frequency of allergic diseases is increasing day by day. Asthma, atopic dermatitis (AD), hay fever, food allergy, eosinophilic esophagitis (EoE), and allergic rhinitis are among the most prevalent chronic NCDs in children from 0-18 years of age [5, 6]. If food allergy accompanies AD in young children, asthma and allergic rhinitis (AR) may develop in these children at later ages. This clinical course is known as the "**atopic march**". Epidemiologic studies indicate that asthma begins in the preschool years even when chronic symptoms do not appear until early adulthood [7, 8]. The lack of definitive diagnostic criteria in differentiating children with recurrent bronchiolitis/bronchitis patients who have progressed to asthma and those who have had one or two non-recurring bronchiolitis/bronchitis attacks prevents predicting the progression of these children. The genes containing differential methylation robustly associated with asthma and allergy are likely to play a role in allergic disease pathogenesis, and understanding the biological implications of these epigenetic signatures may help elucidate mechanistic pathways and define disease endotypes [9].

The diagnosis of allergic disorders is most often made on clinical history, supported by the results of skin tests and diagnostic markers *e.g.* immunoglobulin E (IgE). Although skin testing has advantages of relative sensitivity and specificity, rapid results, and good tolerability, it is subject to some operator, observer and interpretation variability. IgE is also known to be elevated in non-allergic conditions and many allergic patients have IgE levels within the normal range [10]. Schools *et al.* [11] demonstrated that there is a substantial disagreement between skin prick test (SPT) and specific IgE (sIgE) for diagnosing allergic sensitization in young children, which increases with age for food sensitization. sIgE and SPT cannot be used interchangeably as the choice of method significantly influences the diagnosis of IgE - mediated allergy in young children. The lack of agreement between tests and a poor correlation to clinical disease underscores that allergy testing should not be used as a screening tool in children [11].

CHAPTER 18

Biomarkers in Liver Disease

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Abstract: Symptoms and signs of liver diseases are highly variable depending on the etiology, disease stage, and type of liver involvement. There are different types of liver diseases; causes of liver diseases may be viral, toxic, metabolic, or autoimmune. However, in some cases, liver disease can develop as a result of diseases of other organs or systems. It is almost impossible to differentiate all of these solely on the basis of clinical symptoms and findings. Furthermore, the early stages of liver disease may be completely asymptomatic, or in some cases, the disease may progress with only subtle and non-specific symptoms. Therefore, biomarkers have a critical role in screening, diagnosis, staging, and evaluation of therapeutic response to treatment in liver diseases.

Keywords: 5'-Nucleotidase, Alanine aminotransferase, Albumin, Alkaline phosphatase, Alpha-fetoprotein, Aspartate aminotransferase, AST isoenzymes, Biomarkers, CA 19-9, Cholangiocarcinoma, Cholestasis, Fibrosis, Gamma-glutamyl transferase, Hepatocellular carcinoma, Hepatocellular injury, Liver disease, MELD-Na score, Prognosis, Prothrombin time, Staging of liver disease.

INTRODUCTION

Biomarkers are important tools for screening, diagnosis, assessment of treatment response, and evaluating the prognosis of diseases or other medical conditions. There are various definitions for biomarkers in the medical literature, and a more broadened definition of biomarkers may as well include the measurable clinical signs, radiologic or histopathologic findings [1].

National Institutes of Health Biomarkers Definitions Working Group defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic interventions" [2].

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Pınar Atukeren and Hafize Uzun (Eds.)

The burden of liver diseases has been underestimated for many years. Recent data show that liver diseases account for approximately 2 million deaths per year worldwide [3]. Although WHO aims to reduce the incidence of HBV and HCV infections and the associated mortality substantially by 2030, changes in the epidemiology and etiology of liver diseases indicate that nonalcoholic fatty liver disease and related other diseases will be much more important public health problems in the future [4, 5]. Rational usage of biomarkers is not important only for the diagnosis of liver diseases but also for the prediction and prevention of life-threatening complications and other clinical consequences. Unfortunately, most of the biomarkers used for liver diseases in daily clinical practice have some limitations.

The liver may be affected directly and specifically by viral, toxic, or hereditary disease, but also some systemic diseases may cause liver dysfunction or even overt liver disease that is indistinguishable from the liver-specific diseases. Clinical symptoms such as nausea, vomiting, right upper quadrant abdominal pain, fatigue, weakness, weight loss, and jaundice are not specific to liver diseases and may also be seen in many other diseases or medical conditions. Furthermore, these symptoms may not occur until late-stage liver disease. Diagnosis of liver diseases is more difficult than the diagnosis of many other systems' diseases and requires not only a high level of knowledge about liver diseases but also advanced clinical experience.

The intervals between the beginning of the disease, the appearance of the clinical signs and symptoms, and detectable laboratory findings of the liver disease are highly variable; thus, there may be different timeline models for the course of the liver disease (Fig. **1**).

One typical example for model-A is Bud-Chiari syndrome. Immediately after the occlusion of the hepatic veins with thrombus (cause of the liver disease), radiological findings of the venous obstruction can be demonstrated at the early phase of the disease; painful hepatomegaly and ascites (clinical findings) occur within a short time period, followed by other laboratory and clinical findings (Fig. **1A**).

The clinical course of some genetic liver diseases may be described as model-B. Although some of the laboratory findings are present from the beginning or earlier in the course of the disease, clinical findings may be delayed for a long time (Fig. **1B**). Wilson's disease is caused by a mutation in the ATP7B gene; low levels of serum ceruloplasmin and increased urinary copper excretion may be demonstrated many years before overt clinical signs and symptoms.

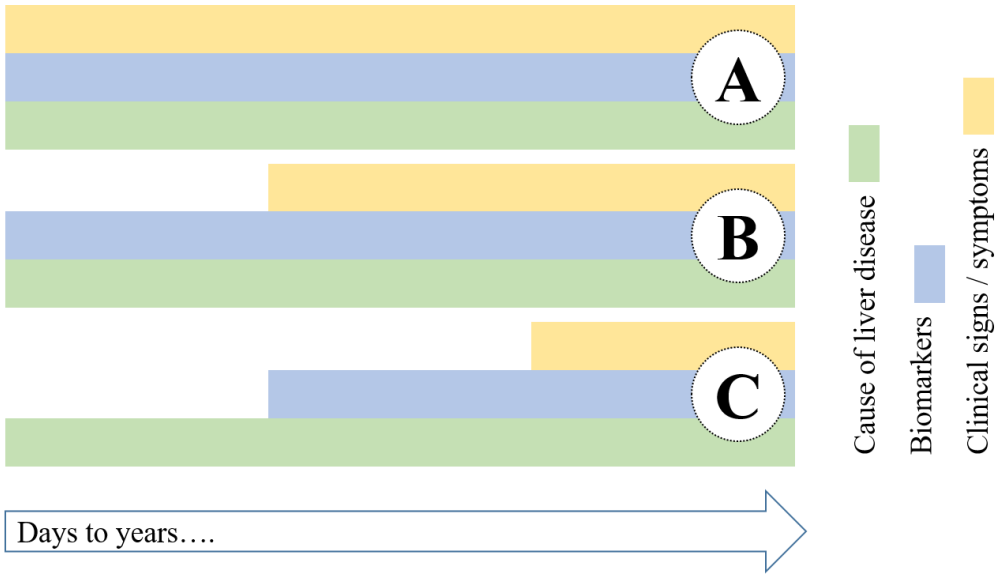

Fig. (1). Different timeline models depicting the manifestation sequence of "The cause", "The biomarker positivity" and "Clinically apparent signs and symptoms" in liver diseases.

Clinical models of some other liver diseases may have a long-time-interval between laboratory and clinical signs of the disease; the cause of the liver disease may persist for many years without any significant alteration of laboratory tests and before clinical symptoms or signs are evident (Fig. **1C**). The course of the immune tolerant phase of chronic hepatitis B virus infection is typical for this model. Despite the initial HBsAg positivity and the presence of high levels of HBV DNA, neither the biochemical findings of liver damage nor the clinical signs of liver disease are present. Aminotransferase elevation will manifest in the next phase (immune active phase), but many years later, clinical signs and symptoms of liver disease will be evident in some of these patients.

Biomarkers are of critical importance for the diagnosis and clinical evaluation of most of the liver diseases in any of the three clinical course models.

Biochemical findings used for the diagnosis of liver diseases are classified into four groups traditionally.

1. Tests used to evaluate the hepatocellular injury
2. Enzymes for detection of cholestasis
3. Tests of liver synthetic function
4. Quantitative liver function tests

CHAPTER 19

Application of New Acute Kidney Injury Biomarkers

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Abstract: Kidney-related biomarkers can provide structural and functional information about different parts of the nephron. These biomarkers can be used to evaluate glomerular, tubular, or interstitial injury, inflammation, or repair, and glomerular or tubular function. Furthermore, biomarkers can improve the acute kidney injury diagnosis in various clinical conditions, including acute interstitial nephritis, acute tubular injury, hepatorenal and cardiorenal syndrome, ischemic and nephrotoxic acute kidney injury, and drug-induced acute kidney injury. Biomarkers might be used as an additional precision medicine tool in managing patients with acute kidney injury; they can help with clinical decision-making and impact patient outcomes. In this chapter, we reviewed the utility of biomarkers used in acute kidney injury.

Keywords: Acute kidney injury, Biomarkers, Nephrotoxicity, Tubular injury.

INTRODUCTION

Acute Kidney Injury, Biomarkers, And Definitions

Acute kidney injury (AKI) is the rapid loss of kidney function and is a serious problem, particularly in patients hospitalized in intensive care units (ICUs) [1, 2]. However, until about two decades ago, the condition lacked a uniform definition. The Acute Dialysis Quality Initiative first introduced the international consensus criteria, which was subsequently modified by the AKI Network and finally by the Kidney Disease Improving Global Outcomes (KDIGO) workgroup [1]. Although a standardization in the definition of AKI has been achieved using the serum creatinine and occasionally urine output, there is still a need for more robust tools to define the etiology, type, and prognosis of AKI [3].

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Pınar Atukeren and Hafize Uzun (Eds.)

The KDIGO guidelines emphasize early diagnosis and treatment in AKI, and the preferred diagnostic biomarker of the condition remains the measurement of the serum creatinine level because of its convenience and low cost. However, the utility of this marker is limited due to many reasons; for example, renal hypoperfusion may cause prerenal kidney injury, and creatinine levels may increase without any renal parenchymal damage [4]. Additionally, in patients with renal parenchymal injury, especially in chronic kidney disease (CKD) patients, renal compensation may delay the creatinine increase, and creatinine levels might not increase until a GFR loss of more than 50% occurs [5, 6]. Therefore, more sensitive and specific new biomarkers will likely improve the early diagnosis of AKI [7].

Biomarkers in kidney injury are new tools for risk assessment that can help determine which therapy to use. [8]. The Food and Drug Administration (FDA) has defined a "biomarker" as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic interventions [3].

Classification of AKI Biomarkers

Different schemes, usually based on the site of origin or pathophysiological process on the nephron (Table **1**) [9, 10], might be used to classify the AKI biomarkers. Biomarkers can help evaluate glomerular or tubular function, glomerular, tubular, or interstitial injury, inflammation, or repair. Selected biomarkers of AKI according to the site of origin on the nephron are shown in Fig. (**1**).

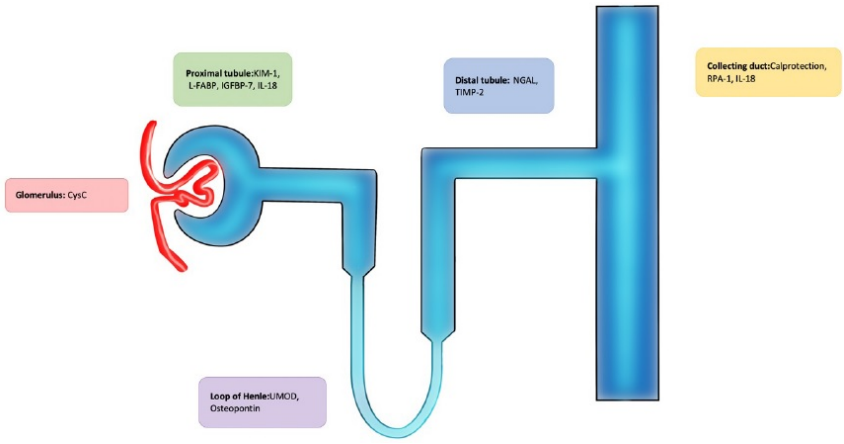

Fig. (1). Biomarkers and their predictive area of injury in the nephron. The illustration was prepared using the following references [10, 75, 94, 98].

Additionally, tissue inhibitors of metalloproteinases-2 (TIMP-2) and insulin-like growth factor binding protein-7 (IGFBP-7) might be classified as cell cycle arrest biomarkers [11].

Table 1. Acute kidney injury biomarkers classified according to the site of origin and pathophysiological process on the nephron [9, 10, 45].

Biomarkers of glomerular health	Cystatin-C, galectin-3, proenkephalin
Biomarkers of tubular health (function and injury)	KIM-1, L-FABP, IGFBP7, TIMP-2, UMOD, NGAL
Biomarkers of inflammation	TNF-alpha, IL-9, IL-18, IL-6, IL-10, TNFR1, TNFR2
Biomarkers of repair and fibrosis	MCP-1, CHI3L1 (YKL 40), PIIINP, EGF

KIM-1: kidney injury molecule-1, L-FABP: liver-type fatty acid-binding protein, TIMP-2: tissue inhibitor of metalloproteinases 2, IGFBP-7: insulin-like growth factor-binding protein 7, UMOD: uromodulin, NGAL: neutrophil gelatinase-associated lipocalin, TNF-alpha: tumor necrosis factor alpha, IL: interleukin, TNFR1: tumor necrosis factor receptor 1, TNFR2: tumor necrosis factor receptor 2, MCP-1: monocyte chemoattractant protein 1, PIIINP: procollagen type III N-terminal propeptide, EGF: epidermal growth factor.

Biomarkers of Glomerular Health

Cystatin C: Classically, creatinine is used as a biomarker of glomerular health, while cystatin C is a new biomarker that depicts the glomerular function. In contrast to creatinine, serum cystatin C is not correlated with age, sex, and muscle mass, and in certain situations, such as in patients with marginal muscle mass, it might be used as a more appropriate marker of glomerular function [12]. Cystatin C is eliminated entirely by glomerular filtration. Following filtration, the protein is almost completely catabolized in the proximal tubule. Therefore, in physiological conditions, it scarcely appears in the urine. An impairment in the proximal tubular reabsorption causes an increase in the urinary cystatin C levels [13].

Biomarkers of Tubular Health

Alpha-1-Microglobulin (α1M): Alpha-1-microglobulin is a biomarker of proximal tubular function and is mainly produced by the liver. The protein might be in free form, or it might form a complex with immunoglobulin A (IgA) [14]. Following free glomerular filtration and proximal tubular reabsorption, α1M is completely catabolized. Increased urinary concentration of this biomarker is associated with proximal tubular injury or dysfunction. Urinary α1M extraction is increased in patients who have a renal tubular disease [14].

Liver-type Fatty acid-binding Protein (L-FABP): There are several types of FABPs expressed in various organs, including the kidneys and the liver. Following glomerular filtration, the circulating L-FABP is reabsorbed by the

CHAPTER 20

Biomarkers to Predict Sudden Cardiac Death

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Abstract: Sudden cardiac death (SCD) is a common disorder and an unsolved issue for health care providers. Despite several risk factors for SCD, some cases experience SCD as an initial presentation of cardiovascular disease. Prediction of individuals at increased risk for SCD is important for implementing community-based approaches and individual-based therapies with high costs, such as implantable defibrillators. This chapter discusses the potential role of biomarkers in predicting SCD in different cardiovascular diseases.

Keywords: Biomarkers, Cardiovascular disease, Health care providers, Implantable defibrillators, Risk factors, Sudden cardiac death (SCD).

INTRODUCTION

Sudden cardiac death (SCD) is defined as the unexpected death of a person from the loss of cardiac function either within one hour of medical observation or 24 hours without medical observation. Cardiovascular diseases are responsible for 17 million deaths every year, and 25% of these are SCD. Males are currently at higher risk in terms of cardiovascular disease. SCD affects 6.68 out of every 100,000 males and 1.4 out of every 100,000 females. The highest prevalence of SCD is amongst individuals with a history of cardiac arrest or myocardial infarction, and this SCD risk is at the highest level between the 6th and 18th months after the initial cardiac event. There are also racial differences observed among the SCD cases; the black race is at more risk than the white race. SCD occurs mostly outside of the hospital or in the emergency rooms. There are different mechanisms identified that lead to SCD. Physical fragility of the atherosclerotic plaque, thrombus formation due to this, and unstable electrical conductivity are amongst the reasons for SCD in individuals with coronary atherosclerosis. Arrhythmic events can also be accelerated by hypokalemia and

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Pınar Atukeren and Hafize Uzun (Eds.)

certain drugs that can alter QT time. Genetic causes of arrhythmogenic cardiac death, such as channelopathies, are amongst the common reasons behind SCD in relatively young population [1 - 5]. This review aims to discuss possible risk factors, especially biomarkers, among different cardiovascular substrates.

SCD in the Setting of an Acute Coronary Syndrome, Ischemic Cardiomyopathy

Sudden death is mostly due to cardiac causes and is mainly seen in patients with heart disease secondary to ischemia. Sudden death due to cardiac causes constitutes approximately 50% of all cardiovascular deaths. STEMIs that lead to SCD are associated with more proximal acute coronary artery occlusion than STEMIs without SCD. Compared to patients with STEMI, NSTEMI patients who develop SCD have been shown to be elderly patients with diffuse and multiple coronary artery disease and have more cardiovascular risk factors [4].

The risk of SCD is higher, especially in the first 30 days after MI. Although its predictive value for myocardial scar formation is weak, an echocardiogram is one of the imaging methods that can predict SCD due to ventricular tachycardia or ventricular fibrillation. As ejection fraction of the left ventricle is an indirect measure of an LV scar and can be affected by hemodynamic-autonomic factors, direct visualization of the scar and its architecture may increase risk stratification accuracy. Cardiac MR imaging with late gadolinium enhancement is superior to echocardiography in determining total scar tissue [6]. The relationship between certain ECG changes has been identified with SCD, either with or without the coronary syndrome. QRS-T angle between depolarization and repolarization, QRS-T area angle, QRS area, QT prolongation, QT dispersion, QRS fragmentation, QRS amplitude, and T wave elevation are independent predictors of SCD on an ECG reading. The angle of the QRS-T area below 112 degrees was associated with the low rate of SCD in 30-month follow-up cases [7]. The presence of microvolt T-wave alternation in patients with MI was shown to be risky in terms of arrhythmic events [8]. It is also shown that the presence of early repolarization pattern in the ECG, especially in the inferolateral leads, in patients who have no previous history of cardiac disease, who have had the first acute coronary event, creates the environment for fatal arrhythmias due to which the risk of SCD is doubled [9]. QRS wave fragmentation seen on the ECG has been associated with heterogeneous activation of ventricles due to myocardial scar in individuals with known or suspected coronary artery disease. The presence of fragmented QRS waves in patients with acute coronary syndrome triggers arrhythmogenic mechanisms and can shorten the process of fatal events [10].

The autonomic nervous system plays a key role in the development of ventricular arrhythmias, especially in patients with a history of MI. Sympathetic stimulation in cardiac disease may accelerate the development of VT or VF. Therefore, autonomic tonus measurements are included in the risk classification. Heart rate variability may occur as a decrease in spontaneous variation secondary to the loss of vagal tone after MI. The association of decreased heart rate variability with both SCD and non-sudden death has been documented. Heart rate turbulence, on the other hand, measures the short-term change in heart rate after ventricular premature beats and is closely related to baroreceptor sensitivity. Unlike baroreceptor sensitivity, heart rate turbulence can be obtained from ECG recordings alone. In addition, the REFINE study revealed heart rate turbulence association with fatal or non-fatal cardiac arrest. Compared to heart rate variability, heart rate turbulence may be more specific for SCD due to VT or VF. Advances in scintigraphical imaging (SPECT / PET) may allow visualization of the cardiac sympathetic function of the left ventricle using norepinephrine analogs (C-meta-hydroxyepinephrine). This may reveal both arrhythmic and non arrhythmic outcomes [6]. In patients presenting acute coronary syndrome symptoms, several biomarkers have been evaluated for endpoints. In a study conducted with NT-proBNP, GDF-15, CRP, cystatin-C, troponin I, and troponin T values, it was shown that basal levels of NT-proBNP and GDF-15 are associated with arrhythmia and SCD [11]. Natriuretic peptides are associated with an increased risk of SCD in both men and women. There is evidence that effective HF treatments reduce BNP values, as in the MADIT-CRT study, and transform into a better prognosis for ventricular arrhythmias and SCD. Patients with decreased BNP levels by more than a third of baseline have a lower risk of VT/VF [12]. Cardiac troponins play an important role in the evaluation of patients with the acute coronary syndrome. Detectable levels of hs-Tn in the circulation reflect cardiac myocyte damage. Basal hs-Tn levels have been shown to be associated with SCD, and it is postulated that detectable levels of hs-Tn may provide an anatomical substrate for scar-related re-entry into ventricular tachycardia. The fact that troponin levels are specific for cardiac myocytes and can be measured more easily than other lethal rhythm predictive biomarkers provide clear advantages to the clinician [13]. Sst-2 is a member of the interleukin-1 family and is associated with myocardial dysfunction and fibrosis. It plays a role in the pathophysiological processes of different ischemic events, including the progression of coronary atherosclerosis. Although serum levels of Sst-2 increase with ischemic damage, they may remain high even after MI. As a result of the analysis obtained from the MUSIC records, Pascual-Dual *et al.* were able to show that high Sst-2 levels are associated with SCD. The authors suggested that the combination of natriuretic peptide and Sst-2 may be valuable in predicting SCD [12]. Cystatin-c, which is one of the indicators of renal dysfunction and plays a role in measuring the degree

CHAPTER 21

Oxidative Stress Biomarkers in the Diagnosis and Prognosis

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Abstract: Oxidative stress describes the state of a cell where there is an imbalance between free radical formation and antioxidants due to either excess formation of reactive oxygen species (ROS) or inadequate antioxidant defence. It is very well known that oxidative stress plays an important role in the pathophysiology of various diseases through impaired intracellular redox homeostasis. To evaluate and imply the excess production of ROS, various biomarkers are used and suggested, yet it is also known that there is a lack of standardization and validation for these methods. It is almost very difficult to measure ROS directly because of their short half-life, yet it is still possible with a suitable technique. The most frequently used biomarkers are represented by oxidized macromolecules such as lipids, proteins, and nucleic acids, which are modified *via* ROS, and also the amounts or activities of antioxidant molecules and enzymes, respectively. There are also various genetic biomarkers measuring the susceptibility of modification due to oxidative stress. However, the preferred biomarker would be dependent on the aim of the study and the clinical relevance.

Keywords: Antioxidants, Oxidative biomarkers, Oxidative stress, Reactive oxygen species.

INTRODUCTION

Oxidative Stress

In normal cells, there is balanced redox homeostasis which is regulated by free radicals' production and the antioxidant defense systems [1]. Reactive oxygen species (ROS), so-called free radical molecules, are produced not only during normal cellular metabolism but also there are various exogenous sources of ROS (Tables **1** and **2**). In both experimental and clinical medicine , oxidative stress,

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which is mediated by ROS due to either an excess of ROS or a reduction in the amount of activity of antioxidant defense mechanisms, is important [2]. ROS are known to have a dual role as they might also have beneficial effects [3]. The intracellular redox imbalance is induced by oxidative stress, which triggers a number of factors such as cancer stimulation, increased metabolic activity, and mitochondrial dysfunction [4, 5]. The cell's redox status includes physiological and pathological effects related to the endogenous antioxidant status, which has a vital role in the prevention against oxidative stress due to the imbalanced defense mechanism of antioxidants, and the healthy living system will be exposed to serious damage [6].

Table 1. Reactive oxygen species.

$O_2^{\cdot-}$ (Superoxide radical)
H_2O_2 (Hydrogen peroxide)
$HO^{\cdot}$ (Hydroxyl radical)
HOCl (Hypochlorus acid)
Singlet O_2
$R^{\cdot}$ (Alkyl radical)
$ROO^{\cdot}$ (Peroxyl radical)
$RCOO^{\cdot}$ (Organic peroxyl radical)
$HO_2^{\cdot}$ (Peroxyl radical)
$RO^{\cdot}$ (Alkoxyl radical)

Table 2. Sources of reactive oxygen species.

Endogenous sources	**Exogenous Sources**
Mitochondrial electron transport chain	Radiation (UV lights, X-rays, Gamma-rays)
Autooxidation reactions	Chemotherapeutics, Chemicals, Drugs
Redox cycles	Environmental toxins
Cytochrome p450 reactions	Diet; Charcohalbroiled foods
Xanthine oxidase	Smoke
NADPH oxidase, Lipoxygenase, Prostaglandin synthase	
Inflammation; Oxidative reactions in phagocytic cells (Myeloperoxidase)	
Transition metals	

Indeed biological oxidation is the natural process of aerobic metabolism, but during this process, these destructive reactive molecules are produced constantly and express deleterious effects on human cells and damage [7].

Antioxidants

When the antioxidant system is unable to neutralise the overly generated ROS, oxidative stress develops as a result of the impaired redox balance [8]. There are many antioxidant defense systems in the human body to cope with the oxidative damaging effects of ROS [9]. The antioxidants might be either endogenous or exogenous too. They might be water or lipid-soluble due to their molecular structure. Some antioxidants are vitamins such as vitamin C and vitamin E; some antioxidants are enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR). SOD is the first defending enzyme dismutating O_2^- . Also, there are some endogenous metabolites having antioxidant activities (Table **3**).

Table 3. Antioxidants.

Enzymatic Antioxidants	Superoxide dismutase (SOD) Catalase (CAT) Glutathione peroxidase (GPx) Glutathione reductase (GR) Glutathione-S-transferase Thioredoxine system (Trx/TrxR)
Non-enzymatic Antioxidants	Glutathione (GSH), Ubiquinone, Selenium Vitamin A, Vitamin C, Vitamin E, α-Lipoic acid, Flavonoids Albumin, Haptoglobin, Ceruloplasmin, Lactoferrin Uric acid, Bilirubin, Melatonin

Cells have various antioxidant mechanisms that balance the concentrations of ROS. SOD, CAT and GPx are the most effective [10]. There are non-enzymatic antioxidants such as vitamin C, vitamin E, carotenoids and glutathione, thioredoxin, lipoic acid, which are thiol antioxidants [11]. Glutathione (GSH) is a key thiol-containing antioxidant that detoxifies intracellular H2O2 to H2O and O2 through GPx and recycles through (GR), which is critical for maintaining cellular redox equilibrium [12, 13].

Oxidative Stress Biomarkers

Oxidative stress status is evaluated by investigating the cell's oxidant and antioxidant status as various biomolecules will be damaged. Oxidative stress biomarkers are related to the status of the disease and the effects of antioxidant molecules. The frequently measured oxidative stress related biomarkers and their properties are shown in Table **4**. These biomarkers can be measured in biological samples, mostly blood and tissue samples and also in other biological fluids such as urine and saliva.

CHAPTER 22

Current Biomarkers for Endometrial Receptivity

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Abstract: Implantation and the continuation of pregnancy occur through a complicated and sophisticated dialogue, called "cross-talk," which starts between the embryo and the endometrium in the early stages of oocyte maturation. This dialogue provides synchronization of the journey of the embryo to be implanted with the receptive endometrium. Understanding the activity and function of the hormones and factors involved in this dialogue will provide an understanding of endometrial receptivity, which plays a key role in implantation, and the determination of biomarkers specific for this period. As a result of the development of omics technology, it has become possible to identify biomarkers specific to endometrial receptivity by performing genomic, proteomic, and lipidomic analyses of these hormones and factors. The determination of these biomarkers, their optimization, and making them usable in the clinic will allow increased success in ART.

Keywords: Biomarkers, Endometrial receptivity, Genomic analysis, Implantation, Lipidomic analysis, Omics, Proteomic analysis.

INTRODUCTION

Endometrial Receptivity

A successful pregnancy is achieved only with successful implantation, with two important factors forming the basis of successful implantation. The first is to obtain a good-quality embryo, and our chance to obtain quality embryos has increased with the development of assisted reproduction techniques. The second is the receptive endometrium [1]. The allowance of the endometrium of implantation of the blastocyst. The receptive endometrium has a very important physiological role in implantation and also in the maintenance of pregnancy. However, the

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Pınar Atukeren and Hafize Uzun (Eds.)

endometrium is not always receptive through the luteal phase of the menstrual cycle, and its time is limited. This short period lasting about 4 days at most in the mid-secretory phase is called the implantation window, which usually corresponds to the 20th-24th day of the cycle [2, 3]. Other than this window, the endometrium is non-receptive. In order to achieve successful implantation, the journey of the embryo and the receptive period of the endometrium must be synchronized with each other [4, 5].

This happens through "cross-talk" between the embryo and the adhesive endometrium by means of endocrine, paracrine, and autocrine factors [6].

The mechanism for the non-receptive endometrium to become receptive is not fully understood. Studies have determined that endometrial receptivity occurs due to the development of changes such as adhesion, invasion, growth, differentiation, decidualization, and immunomodulation. The factors arising during these developing changes play an important role in the preparation process for implantation [7, 8].

Various studies have been conducted to determine these synthesized factors in order to better understand endometrial receptivity. These studies have been conducted with a wide range of endometrial sampling for the use of omics technology [6, 9].

Determining the activity and function of these factors in the dialogue between the embryo and the endometrium allows us to use these factors as endometrial receptivity biomarkers in the clinic. Considering that two-thirds of recurrent implantation failure (RIF, *i.e.*, failure of three IVF cycles in which one or two high-quality embryos are transferred to the patient per cycle) is due to endometrial receptivity; it is very important to determine potential biomarkers and evaluate their significance in order to provide utility in the clinic and increase the success of implantation [1, 10].

Markers Brought by New Technology

The complete sequencing of the human genome has opened a new era of system biology, called omics technologies. The term omics is expressed as a comprehensive analysis of biological systems. The omics technologies driving these new fields of research include DNA and protein microarrays, mass spectrometry, and many other tools that enable high throughput analysis. Due to these developments, it is possible to examine the genome, transcriptome, proteome, and metabolome of cells, tissues, and organisms and to investigate the interactions between them [11, 12].

The histology of the endometrium has been studied since the 1950s to evaluate the developmental cycle and receptivity of the endometrium [9]. When the endometrial surface was examined with electron microscopy, it was determined that new projections were formed on the endometrial surface where the embryo first came into contact. Pinopodes, which were first defined by Psychoyos, were determined by Niklas in a certain period of the cycle, namely in the implantation window, and were named as endometrial receptivity markers [13, 14]. The development of omics technology, genomic, proteomic, and lipidomic studies have enabled the search for new biomarkers that can evaluate endometrial receptivity and provide utility in the clinic. Diagnostic markers of the implantation stage can be found by examining the microarray of many genes expressed in the endometrium at the stages of the menstruation cycle. Many proteins related to reproduction can be discovered by proteomic analysis. Thus, the detection of biomarkers specific to the receptive and non-receptive period of the endometrium could be identified [15].

Genomic Study of the Endometrium

In recent years, many transcriptomic studies in which important information has been obtained in determining the receptive endometrium have been conducted. These studies have found 107-2878 genes that are expressed differently in the endometrium by up and down-regulation during the transformation of the endometrium from the pre-receptive (LH+1/5] period to the receptive period (LH+7/9] [5, 16, 17]. However, the results have not shown a correlation with each other due to the different microarrays used for the analysis and the different sampling methods and conditions in these studies. For this reason, a common consensus could not be reached in the transcript studies. Another issue that causes confusion in these studies is whether the evaluation of the endometrium should be based on the natural cycle of fertile women or the cycle of infertile women. The studies conducted to solve this problem are classified into three groups: 1) the comparison of fertile women with the cycles of infertile women, 2) studies on the natural cycles of fertile women [16, 18 - 20] or fertile donors [21,22], and 3) the comparison of natural cycles with stimulated cycles [21]. Having such a classification in studies will facilitate the determination of biomarkers of the non-receptive endometrial period (proliferative or early secretory) and the receptive endometrium (mid-secretory), and will enable the determination of common biomarkers from the results of the studies on the cycles, increasing the specificity of the biomarkers.

In 2011, Diaz-Gimeno *et al.* identified 238 genes with expression specific to early and mid-secretory phases, 134 of which carry transcriptomic signatures of the

CHAPTER 23

Biomarkers for Preterm Delivery

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Abstract: Preterm birth occurring before the thirty-seventh gestational week complicates 4.5%-18% of pregnancies worldwide. The pathogenesis of spontaneous preterm delivery is not fully understood. Among the factors held to be responsible for its pathogenesis, the most emphasized is the inflammatory process. Studies in terms of the prediction of preterm delivery are basically divided into 3 categories: 1) Prediction in pregnant women who are asymptomatic and without risk factors, 2) Prediction in pregnant women who are asymptomatic and have risk factors, 3) Prediction in symptomatic pregnant women who have threatened preterm labour. In this chapter, the topic of biomarkers in relation to preterm delivery is discussed. The most commonly used markers in published studies are fetal fibronectin, cervical pIGFBP-1 and cervical length measurement by transvaginal ultrasound. For prediction in symptomatic pregnant women applying to the hospital with threatened preterm labour, the markers used are fetal fibronection, insulin-like growth factors (IGFs) and inflammatory markers. Preterm labour prediction with markers checked in the first and second trimesters are fetal fibronection, insulin-like growth factors (IGFs), micro RNAs, progesterone, circulating microparticles (CMPs), inflammatory markers, matrix metalloproteinases, aneuploidy syndrome screening test parameters and other hormones.

Keywords: Aneuploidy syndrome screening test parameters, Circulating microparticles, Corticotropin-releasing factor, Corticotropin-releasing hormone, C-reactive protein, Fetal fibronectin, Hsa-let-7a-5p, Hsa-miR-15b-5p, Hsa-mi--19b-3p, Hsa-miR-23a-3p, Hsa-miR-93-5p, Hsa-miR-150-5p, Hsa-miR-185-5p, Hsa-miR-191-5p, Hsa-miR-374a-5p, IGFs, IGFBPs, Inflammatory markers, Insulin-like growth factor-binding protein 4, Interleukin 6, Lipopolysaccharide-binding protein, Macrophage inflammatory protein, Matrix metalloproteinases, MicroRNAs, PAPP-A, Placental alpha microglobulin-1, Prediction, Preterm birth, Preterm delivery, Toll-like receptors.

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Pınar Atukeren and Hafize Uzun (Eds.)

INTRODUCTION

Preterm Delivery

Preterm birth occurring before the thirty-seventh gestational week complicates 4.5%-18% of pregnancies worldwide [1 - 3]. Preterm birth can occur in three ways: 1) Spontaneous preterm delivery without preterm premature rupture of membranes, 2) preterm delivery after preterm premature rupture of membranes, 3) Preterm labour induced by maternal or fetal indications. This chapter aims to cover spontaneous preterm labour without preterm premature rupture of membranes. Preterm delivery occurs as a result of 40-50% spontaneous preterm labour, 25-30% after preterm prelabour membrane rupture and 30-35% medical or obstetric reasons [4]. Spontaneous preterm delivery is one of the most important causes of neonatal mortality and morbidity [5].

The pathogenesis of spontaneous preterm delivery is not fully understood. Among the factors held to be responsible for its pathogenesis, the most emphasized is the inflammatory process [6 - 8]. Spontaneous preterm delivery occurs as a result of the onset of labour after pathological signal transmission [8]. The onset of labour begins and progresses with similar mechanisms in both term and preterm pregnant women. All mechanisms cause cervical maturation and initiation of uterine contractions [9]. Cervical maturation is associated with an increase in prostaglandins, chemokines and cytokines, and infiltration of inflammatory cells and increased matrix metalloproteinase activity [10]. Spontaneous delivery occurs from proinflammatory cytokines in the myometrium, amniotic membranes and cervix, and through increased uterine contractility and cervical dilatation due to the increase in prostaglandins [11].

The onset of uterine contractions before the 37th gestational week is called threatened preterm labour (TPL). 9% -24% of pregnant women are hospitalized due to TPL [12 - 15]. However, spontaneous preterm delivery occurs in approximately 50% of pregnant women with complaints related to TPL [15].

Prediction of which women in preterm labour will have a preterm delivery would enable us to apply tocolysis and antenatal steroids to the right patients. Also, this would make it possible for us to refer the mother to a tertiary maternity centre, for the mother to give birth and the baby to be born under safer conditions [16]. In addition, pre-determination of women who will have preterm delivery, and referral of risky pregnancies to perinatal centres and corticosteroid therapy may be possible [17]. There are still no markers with high sensitivity and specificity that can be used for this purpose [18 - 20]. Women who had a preterm birth in their previous pregnancy are in the high-risk group in terms of preterm delivery in their next pregnancy. However, most women with preterm birth do not have a history

of preterm birth [21]. Therefore, there is a need for markers to predict preterm birth, except for those with a history of preterm birth.

Studies in terms of the prediction of preterm delivery are basically divided into 3 categories: 1) Prediction for pregnant women who are asymptomatic and without risk factors, 2) Prediction for pregnant women who are asymptomatic and have risk factors, 3) Prediction for symptomatic pregnant women who have threatened preterm labour. These 3 categories will be examined under separate subheadings.

Prediction in Symptomatic Pregnant Women Applying to the Hospital with Threatened Preterm Labour

The most commonly used markers in published studies are fetal fibronectin, cervical pIGFBP-1 and cervical length measurement by transvaginal ultrasound [22].

a). Fetal Fibronectin

Fetal fibronectin (FFN) is found in maternal choroidal tissues, and with the inflammatory process, it is separated from the tissues and mixed with cervicovaginal tissue [23]. The quantitative fetal fibronectin (qFFN) measured in the cervicovaginal fluids of pregnant women who went to the hospital due to preterm labour had a high negative predictive value in predicting preterm labour, but its positive predictive value was lower [24 - 26]. Iams *et al.* reported that fetal fibronectin (in cervicovaginal fluid) may be useful in determining the birth that will develop within 7 days for those who go to the hospital with threatened preterm labour [27]. In their large series prospective study, Esplin *et al.* reported that quantitative vaginal fetal fibronectin and serial transvaginal ultrasound cervical length measurement have low predictive value in predicting spontaneous preterm birth in patients with a nulliparous singleton pregnancy, and therefore cannot be recommended for routine use [28]. In the conducted studies, the sensitivity and positive predictive value of cervicovaginal fetal fibronectin measurement in predicting preterm labour were found to be low [29 - 32].

b). IGFBPs

Insulin-like growth factors (IGFs) play a role in fetal and placental development [33]. In circulation, IGFs bind to IGFBP-3. The clearance of IGFs slows down through this binding [34]. Eroglu *et al.* were able to predict preterm deliveries before 35 weeks of gestation by examining insulin-like growth factor binding protein-1 (phIGFBP-1) in cervicovaginal secretions in 90 pregnant women going

CHAPTER 24

Biomarkers in Obesity and Clinical Applications to Surgical Practice: From Pharmacogenomics to Surgigenomics

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Abstract: Personalized medicine is an evolving field and helps in detecting putative targets for pharmacological treatment modalities in obesity treatment. Obesity has become a pandemic and major threat, increasing all causes of mortality and bringing a heavy cost burden on health budgets. Although bariatric/metabolic surgery justified its role as the most effective treatment regimen for obesity and related comorbidities in the long-term, some patients require revisional operations, do not respond to this treatment option, or experience a relapse of their co-morbidities. For this reason, detection of possible non-responders and prediction of patients at high risk for developing cardiovascular or metabolic adverse events in long-term follow-up is of utmost importance. Therefore, the individualization of surgical treatment by the use of novel biomarkers has come into prominence recently. Many biomarkers and gene loci had been identified related to adiposity, insulin resistance, and oxidative stress. In this brief chapter, the recent advances and clinical implications of these biomarkers will be elaborated.

Keywords: Bariatric surgery, Biomarkers, Obesity, Oxidative stress, Inflammation, Personalized medicine, Proteomics.

INTRODUCTION

Obesity has become a global health challenge, affecting almost 27.5% of adults and 47.1% of children worldwide [1]. Obesity increases the development of many chronic diseases such as Type II diabetes (T2DM), hypertension, coronary heart disease, and certain types of cancer, decreasing the quality and life span of an individual. Moreover, obesity has shown to be a multifactorial, heritable disease for which no gold-standard treatment or preventive strategy has been developed [2]. World Health Organization defined obesity as an abnormal or excessive

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Pınar Atukeren and Hafize Uzun (Eds.)

accumulation of fat that impairs the health status of an individual. Diagnosis of obesity has shown variation methodology, and certain indexes, most commonly body mass index and anthropometric measurements, are currently being used as simple methods for the classification of obesity [3].

Bariatric surgery proved its role to be the most effective treatment modality for the long-term management of overweight, obesity, and related comorbidities. Currently, a variety of operations have been approved by international organizations for the treatment of obesity and metabolic disorders; however, there have been no classification criteria developed on which operation should be used for specific patients. It is unfortunate that even today, the decision-making process for the type of operation for the treatment of obese patients depends on the expertise of the surgeon and the patient's choice. It has been well known that certain types of operations yield better results in patients with T2DM and dyslipidemia. There are many comparative retrospectives and prospective studies in the literature regarding the potency and the long-term results of the bariatric procedures; however, randomized trials with concrete evidence are scarce. Also, hundreds of biomarkers have been defined in literature as a result of animal and human studies, but only a couple of these are used routinely in our daily practice.

Identification of predictors of diabetes remission and the markers that reveal the early start of serious complications such as endothelial injury and oxidative stress resulting from diabetes is immensely important for the decision of treatment strategy in metabolically ill patients. Currently, the ability to do whole genome sequencing allows us to detect major genetic polymorphisms that may leave the patients vulnerable to metabolic disorders. Also detecting putative genetic targets for pharmacological therapy in obese and diabetic patients may help the development of drugs that can show a similar mechanism of highly effective bariatric surgical procedures [4].

Genetic variations among patients treated with bariatric surgery might provide clues for differences in outcomes. Patients with single-gene mutations that lead to early-onset monogenic obesity were recently found to be more resistant to weight loss induced after sleeve gastrectomy. This fact might explain the presence of non-responders after revisional surgical procedures, that who cannot accomplish satisfactory weight loss. Thus, in this chapter, we tried to make a thorough evaluation of the biomarkers that can be used for the decision-making process for the treatment of obesity and co-morbidities [5].

Importance of Personalized Medicine

Up-to-date evidence implies that patients may show variable responses to medical and surgical treatment of obesity and metabolic disorders. Yet every patient has a

unique pharmacogenetic profile. This genetic variability determines the outcomes of both primary and revisional bariatric surgical procedures. It has been evident that pharmacotherapy alone cannot accomplish optimal results compared to surgery. However, it is known that even the most effective methods of bariatric surgical practice may fail in the long-term resulting in weight recidivism and relapse of co-morbid disorders. This is why detecting genetic mutations that may lead to this failure before the operation may help decide on the best procedure tailored for the individual patient [6, 7].

Personalized health risk assessment and a greater understanding of the specific obesity and co-morbid conditions of an individual may help providers to make more rational and specific medical decisions and may prescribe personalized interventions that will best prevent obesity and associated diseases, delay their onset, or reduce their negative impact on the individual. This fact also is amenable to surgical decision-making strategy. This is why we created the word surgigenomics which means the use of the genetic makeup of an individual to decide on the optimal surgical treatment method for the optimal care of an illness. Also, studying biomarkers in obesity and bariatric surgical practice may increase the number of drugs that can be used for obesity treatment. The best example of this fact is the novel use of liraglutide, which is a synthetic form of glucagon-like peptide-1 (GLP-1). Thus, both the gastric bypass procedure and the use of liraglutide reduce gastric emptying, increase satiety, and decrease food intake. In long-term follow-up, patients with weight regain on this medication showed promising results; personalized medicine can predict patients who might respond to surgery and/or medical treatment modalities and thus provide cost-effective strategies [8].

The Impact of Genes on Obesity Treatment

The impact of genes on obesity is complex, and since obesity is a multifactorial disease, the predictive ability to genotype in bariatric surgery can only be of value with clinical correlation with outcomes. Swedish Obese Subjects study (SOS) is the most prominent non-randomized, prospective trial studying intentional weight reduction in severely obese health effects. As a continuation of this study on patients in the surgical intervention group during 6 years of follow-up with full genomic and bodyweight data, single nucleotide polymorphisms (SNAP) has been studied on 11 obesity candidate genes. Twelve SNPs in the ADIPOQ, FTO, LEP, LEPR, MC4R, PPARGC1A, and TNF gene loci were found to be nominally associated with maximum weight loss after bariatric surgery. However, the correctional analysis showed that only the FTO SNP rs16945088 remained statistically associated with maximum weight loss. Although more than 20 culprit genes are associated with obesity, less than 5% of obese subjects carry these

CHAPTER 25

Clinical Application of New Possible Biomarkers in the Assessment and Monitoring of Nutritional Status

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Abstract: Nutrition is directly related to human health. It is very critical to determine the nutritional status to prevent or diagnose diseases and create the right treatment plans. The determination of the nutritional status provides an early diagnosis of growth and development retardation such as malnutrition. It also plays a major role in preventing diseases that may be caused by vitamin and mineral deficiencies. It helps in the surveillance of one of the world's most serious health problems, namely "obesity." Different ways can be used to assess nutritional status. One of the best ways to assess the nutritional and health status is to use biomarkers. A biomarker is a substance whose detection indicates a specific disease state or a response to a therapeutic intervention. Biomarkers are used to detect nutrient consumption and deficiencies as early as possible, enabling early intervention for metabolic problems. Biomarkers also allow the visualization of diseases that a person might develop or potentially have with a sample, such as blood, tissue, and urine, from the person. Health interventions such as nutritional advice will preserve health or promote rapid recovery. In this chapter, the topic of biomarkers related to nutrition and nutrient deficiencies is discussed. The existence of new possible biomarkers is also reviewed.

Keywords: Biomarker, Dietary biomarkers, Food Exposure Markers, Macronutrient, Metabolomics, Micronutrient, Nutrient, Nutrigenomics, Nutrition, Nutritional Assessment, Omics technologies.

INTRODUCTION

Nutrition and Health

According to World Health Organization (WHO), health is a state of complete physical, mental, and social well-being [1]. In a healthy life, nutrition is an important key factor. Nutrition is also a part of healthy growth and development.

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Pınar Atukeren and Hafize Uzun (Eds.)

The body, as a living organism, needs nutrients to perform mental and physical activities [2].

Nutrition is a compulsory human behavior for survival. Food consumption and hormonal control mechanisms such as hunger and satiety involve many physiological processes. However, this needs to be a conscious behavior to protect and improve one's health and the quality of life. Healthy nutrition is defined as adequate and balanced nutrient consumption, with macronutrients and micronutrients consumed according to personal requirements. These recommended amounts can change according to age, gender, preferences, and physical activity status [3, 4].

Nutrients provide the required "energy" to the body. This energy comes from carbohydrates, proteins, and fats, which are called macronutrients. Micronutrients such as vitamins and minerals do not provide energy but play major roles in the processes of energy metabolism. These include chemical reactions that maintain tissues, electrical conduction of nerves, mechanical workings of muscles, and heat generation to keep body temperature stable [5]. Healthy nutrition is also based on a variety of nutrients. Daily requirements of energy and nutrients are taken into the body with foods. Foods are divided into five groups according to the similarity of nutrients they contain. These are vegetables, fruits, grains, proteins, and dairy products that need to be consumed daily [3].

The nutritional status is stable when a person's nutrient intake meets the requirements. Determination of the nutritional status may prevent diseases and promote health. Most chronic diseases show up due to poor nutrition. Assessing the nutritional status will help determine the lack of nutrients. Early diagnosis of diseases also prevents severe deficiencies and functional disorders. As an intervention program, nutritional support and counseling help improve insufficient nutrition and poor nutritional status [6]. A sufficient and balanced diet, including macronutrients and micronutrients, is beneficial for complete health and, ultimately, the quality of life, especially for patients with chronic diseases/wounds [7].

Malnutrition, such as undernutrition, overnutrition, or particular nutrient deficiencies, are identified during the nutritional assessment. These problems are usually caused by poor or overnutrition. Lack of essential nutrients causes various diseases. Malnutrition problems such as obesity, malnourishment, or diseases caused by nutrient deficiencies are examples of an unhealthy nutritional status [8].

Assessment of Nutritional Status and Methods

The nutritional statuses of people are evaluated by dietitians who are nutrition

experts. However, it requires a multidisciplinary screening to determine the nutritional status. As a result of the evaluation, the requirements and deficiencies are seen, and the possible risks are revealed [9].

The nutrition care process has 4 steps: 1) assessment of nutritional status, 2) identifying the nutritional diagnosis, 3. interventions such as education, supports, counseling, *etc*. 4. monitoring and evaluation of the effectiveness of the interventions [6]. To determine the nutritional status, many methods may be used. Dietary indicators, anthropometric indicators, laboratory indicators, and clinical indicators help to define health problems.

The most harmful nutritional status is malnutrition. It refers to deficiencies, excesses, or imbalances in energy and/or nutrient intake. Early diagnosis of malnutrition can both prevent the long-term treatment of the person and contribute greatly to the national economy. Nutritional assessment methods (such as anthropometric measurements) can easily identify malnutrition. The early detection of malnutrition can save the infant's life [10, 11].

In addition to continuous and regular follow-up, a good nutrition plan is required in the treatment plan. This plan must be constantly evaluated and its usefulness measured. Generally, the ABCD nutrition assessment method is used. It includes *anthropometric (A)* indices based on body measurements, *biochemical* indicators (B), an examination of *clinical* status (C), and *dietary* assessment (D) [12, 13].

Anthropometric

Anthropometry investigates the measurements of the human body. Anthropometric measurements include measurement methods used to evaluate body components such as bone, muscle, and fat tissue [14, 15]. Anthropometry has an important role in the nutritional evaluation and is used in general nutritional screening, surveillance, and follow-up, as well as clinical use. A single measure may not provide a comprehensive overview of the patients' condition and the other measurements combinations are required for certain assessments [16, 17].

Anthropometric measurement components vary according to the age, gender, ethnicity, status (pregnancy, athletes, *etc.*) of individuals. The basic elements of anthropometry are height (length for newborns), weight, body circumferences (waist, hip, and limbs), and skinfold thickness. The cut-off points and the classifications of these measurements depend on the person's status [15, 18].

Body mass index (BMI), a person's weight (in kilograms) divided by the square of his/her height (in meters). It is kg/m^2. WHO classification for BMI is generally

CHAPTER 26

A Physiological Approach to Inflammatory Markers in Obesity

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Abstract: Obesity is one of the most critical health problems all over the world; it is associated with metabolic dysfunction and overnutrition. Changes in the physiological function of adipose tissue, leading to altered secretion of adipocytokines, inflammatory mediators release, and chronic low-grade inflammation, are seen in obesity. Macrophages, neutrophils, CD4+ and CD8+ T cells, B cells, natural killer T (NKT) cells, eosinophils, mast cells, and adipocytes are involved in the inflammatory response that occurs during obesity. Various inflammatory markers are released from these cells. In this chapter, we will mention inflammatory mechanisms and markers of obesity.

Keywords: Adipocytes, Adiponectin, Apelin, Cardiotrophin-1, Ceramide, CfDNA, Fetuin-A, Galectin-3, GlycA, HIF-1α, Interleukin-1, Interleukin-6, Leptin, MCP-1, Omentin, TNF- α, TWEAK, Vaspin.

INTRODUCTION

Obesity is abnormal or excessive fat accumulation. As a result of the excess of macronutrients in adipose tissues, inflammatory mediators such as leptin, interleukin (IL-6), tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), resistin are released, and adiponectin production is reduced. In this way, a state sensitive to the inflammatory process occurs [1]. It is unclarified how obesity-induced inflammation in adipose tissue (AT) is triggered. However, potential mechanisms include dysregulation of fatty acids homeostasis, increased adipose cell size and death, local hypoxia, mitochondrial dysfunction, ER stress, and mechanical stress [2]. These are thought to link chronic caloric excess and AT inflammation or factors that may perpetuate chronic tissue inflammation (Fig. **1**) [3].

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Pınar Atukeren and Hafize Uzun (Eds.)

Numerous immune cell types are involved in the inflammatory response during obesity. These are macrophages, neutrophils, CD4+ and CD8+ T cells, B cells, natural killer T (NKT) cells, eosinophils, and mast cells. They can regulate inflammation either positively or negatively [4].

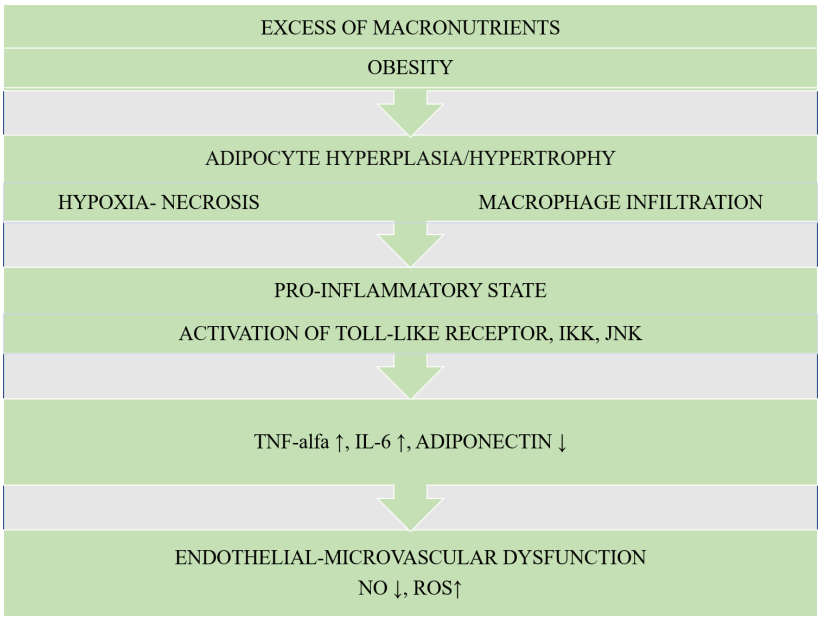

Fig. (1). Pathogenesis of obesity.

Macrophages

Tissue macrophage populations comprise a heterogeneous group of cells, and they have a variety of phenotypes and functions. Mantovani *et al.* defined polarized macrophages in two classes; M1 and M2. M1 macrophages (integrin CD11c +), are proinflammatory, whereas M2 macrophages, which do not express CD11c, are anti-inflammatory [5]. In the obese WAT (white adipose tissue), proinflammatory M1 macrophages form crown-like structures around dead adipocytes, contributing to the obesity-induced, low-grade inflammation. The elevated M1 macrophages in the obese WAT are the main sources of TNFα and IL-6 [6].

Neutrophils

Within a week of starting a high-fat diet (HFD), an increase in neutrophil uptake into adipose tissue occurs, suggesting that neutrophils may play a role in initiating the inflammatory cascade in response to excess nutrients and energy [7].

B Cells

Shortly after initiating an HFD, B cells accumulate in adipose tissues [8]. In addition, the transfer of IgG antibodies from obese WT mice to B cell-deficient mice enhances TNFα production and proinflammatory macrophage polarization [9].

$CD8^+$T Cells

$CD8^+$ T cells are recruited to obese adipose tissue, which begins within two weeks of HFD feeding [10]. Obesity is associated with an increase in the number of CD8+ T cells in AT, and these cells promote macrophage differentiation and chemotaxis [11].

CD4+ T Cells: T-Helper and T-regulatory Cell Subsets

CD4+ T cells can be separated into distinct subsets with diverse phenotypes and functions referred to as T-helper (Th1, Th2, and Th17) cells and T-regulatory (Treg) cells, and each of these subsets is present in AT [12].

Treg and Th2 cells predominate in lean AT, where they prevent the onset of inflammation [13].

Natural Killer T Cells

NKT cells are primarily responsible for target cell killing and cytotoxic activity, but they are also a source of pro-inflammatory cytokines and chemokines. They are present in significant proportions in lean WAT and the liver, although their numbers are depleted after the onset of obesity [14, 15].

Eosinophils

Eosinophils accumulate in adipose tissue by an integrin-dependent process. This promotes M2 macrophage polarization by the secretion of IL-4- or IL-13 [16].

Mast Cells

Mast cells are mainly associated with allergic response [17]. Increased numbers of mast cells are found in obese adipose tissues of mice and humans compared with their lean counterparts, and this is accompanied by increased circulating levels of the mast cell protease tryptase. Genetic depletion of mast cells or pharmacologic inhibition of mast cell function reduces body weight gain, reduces the levels of inflammatory cytokines, chemokines, and proteases in serum and adipose tissue, increases energy expenditure, and improves glucose homeostasis [18].

CHAPTER 27

Application of Biomarkers in the Diagnostic Distinction of Bacterial and Viral Infections

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Abstract: Infectious diseases, which pose a great threat worldwide, have a significant impact on public health and the world economy. It contributes to increased healthcare costs, unnecessary drug-related side effects, and increased antimicrobial resistance. It is not always easy to distinguish the etiological differentiation of diseases that can develop with bacteria and viruses. Therefore, one of the biggest challenges in medicine is how to correctly distinguish between the different causes of these infections and how to manage the patient. Because bacterial and viral infections often present similar symptoms. The real decision is whether the infection is caused by bacteria or viruses and whether to treat the patient with antibiotics. There are many different methodological approaches to diagnosing infections. Biomarkers have been used in the diagnosis of diseases and other conditions for many years. Biomarkers are molecules found in blood and body fluids in measurable amounts, which can evaluate biological and pathological processes. These key indicators can provide vital information in determining disease prognosis, predicting response to treatments, adverse events and drug interactions, and identifying key risks. An effective biomarker is extremely important for the early diagnosis of various diseases. The explosion of interest in biomarker research is driving the development of new predictive, diagnostic, and prognostic products in modern medical practice. The purpose of this review is to demonstrate the use and diagnostic potential of current and investigational biomarkers in the distinction between bacterial and viral infections.

Keywords: Current and investigational biomarkers, Bacterial and viral infections.

INTRODUCTION

Infectious diseases are defined as clinical diseases that occur as a result of many inflammatory reactions in the host when an infectious agent such as bacteria, virus, parasite, and fungus enters the body and multiplies. It can be transmitted directly or indirectly from person to person under appropriate conditions, causing epidemics and pandemics [1].

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The most of emergency infections are caused by pathogens that are already present in the environment or that gain a selective advantage as conditions change, allowing them to infect new host populations. In rare cases, a new variant may also develop and cause a new disease. Emergency infections are a concern, especially when they are spreading rapidly and death rates are high. Previously unidentified or unknown pathogens may be agents that affect new geographic regions or new populations or re-emergent agents with significantly reduced disease incidence in the past. The world is still struggling with various infectious diseases such as malaria, H1N1, Ebola, dengue Fever, Chagas disease, yellow fever, West Nile, Japanese encephalitis, and chikungunya [1].

Gram-negative bacteria caused by newly evolved organisms such as multidrug-resistant tuberculosis and plasmid-mediated colistin-resistant, newly emerging organisms in the human population, HIV-1 infection, respiratory syndrome (SARS) coronavirus in 2003 and MERS-CoV (Middle East respiratory disease) The current coronavirus disease-2019 (COVID-19) pandemic caused by the eastern respiratory syndrome caused by the syndrome coronavirus, and the new coronavirus SARS-CoV-2, which affects the whole world, is challenging the world [2]. Globally, as of October 17, 2020, the COVID-19 outbreak has spread to more than 200 countries and 39,023,292 confirmed cases of COVID-19, including 1,099,586 deaths, have been reported by WHO [3].

With the ongoing evolution of viral and microbial variants and the choice of drug resistance, it appears that new infections will continue to emerge, possibly increase, and will make human beings very challenging. Infectious diseases contribute to increased healthcare costs, unnecessary drug-related side effects, and increased antimicrobial resistance. These diseases have a significant impact on public health and the world economy. In cases where the focus of infection cannot be effectively eliminated or treated appropriately, sepsis, septic shock, and multi-organ failure may develop. In the presence of infection in any part of the body, the immune system begins to react and sepsis occurs due to the body's exaggerated response to this infection. If early treatment is not initiated, organ failure, shock, and death are inevitable. It is not always easy to distinguish the etiological differentiation of diseases that can develop with both bacteria and viruses. Bacterial and viral infections usually present with similar symptoms. In most cases, the decision is whether the infection is caused by bacteria or viruses and whether to treat the patient with antibiotics. This is primarily a challenge for clinicians and healthcare administrators. In addition, correct separation is important in reducing unnecessary antibiotic use [4].

There are many different methodological approaches to diagnosing infections. It

is based on patient symptoms, laboratory tests, radiological examinations as well as clinical findings. These procedures take a long time and are limited. Therefore, additional tools are needed to increase diagnostic accuracy. Biomarkers have been used for many years in the diagnosis of diseases and other conditions. Biomarkers are structures found in the blood or other body fluids, with little or no normal state, that appear or increase in the disease state. Protein metabolites are often used as biomarkers. Nucleic acids and various analytical targets are also used as biomarkers. A better understanding of disease mechanisms is required to fully utilize biomarkers. Bacteria and viruses activate or block different signaling pathways in the cells they infect. Because biomarkers are molecules that can evaluate biological and pathological processes and can be found in measurable amounts in blood and body fluids, they are considered physical, functional or biochemical markers of disease processes. Biomarkers used in the diagnosis of sepsis may play a role in early diagnosis, risk stratification, evaluation, and prognosis prediction. These key indicators can provide vital information in determining disease prognosis, predicting response to treatments, adverse events and drug interactions, and identifying key risks. An effective biomarker is extremely important for the early diagnosis of various diseases [5]. However, the problem with existing biomarkers; It gives late results, low specificity, complicate clinical decisions, and interact with corticosteroid and immunosuppressive treatments. For this reason, new biomarkers are needed to make early and etiological diagnosis, decide on early, correct and adequate treatment, determine the severity of the infection, and evaluate the response to treatment. The explosion of interest in biomarker research will enable the development of new predictive, diagnostic, and prognostic products in modern medical practice. This could advance the use of personalized medicine in the treatment of infectious disease and will also raise important considerations for validating the use of biomarkers as diagnostic or prognostic markers. Many different classes of molecules have been studied in the search for new diagnostic biomarkers to overcome the problems in currently used methods that could help distinguish between bacterial and viral infections. The purpose of this review is to demonstrate the use and diagnostic potential of current and investigational biomarkers in the distinction between bacterial and viral infections.

STANDARD BİOMARKERS

White Blood Cell Counts

White blood cell counts, especially differences in white blood cell counts or neutrophil counts in the blood, are the most commonly used tests to diagnose acute infections. Counting can easily be done manually under the microscope or with automatic cell counters. However, at least 400 cells must be counted for

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Z

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Hafize Uzun

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