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Review Article

BIOMARKERS: AN IMPERATIVE ACCESSION FOR DIAGNOSIS OF A DISEASE AND DRUG DEVELOPMENT

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ABSTRACT

Biomarkers are becoming an essential part of clinical development, not least because they offer a faster alternative to the conventional drug development approach and the promise of safer drugs, in greater numbers, approved more quickly. Many of the failures happen late in clinical trials, with the consequence that expenditure in clinical drug development – already a mammoth effort requiring a huge amount of money, time, and patient is increasing. The ultimate vision is to have access to therapeutic fields, a better understanding of pathophysiology of diseases, thereby uncovering potential drug targets and biomarkers in the disease pathway. By finding molecular biomarkers of the disease, diagnosis could be improved and could reveal new information about the disease, by which a better chance for developing drugs is possible. Biomarkers can also reflect the entire spectrum of disease from the earliest manifestations to the terminal stages. In this present review, biomarkers of various diseases were enlisted to highlight the overabundance of information necessary for clinicians and scientists to have a thorough understanding of biomarkers and its ability to improve treatment and reduce health-care costs which are potentially greater than in any other area of medical research.

Keywords: Biomarkers, Disease, Pathophysiology, Therapeutics, Treatment.

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INTRODUCTION

In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [1,2]. A joint venture on chemical safety, the International Programmed on Chemical Safety, led by the World Health Organization (WHO) and in coordination with the United Nations and the International Labor Organization, has defined a biomarker as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" [3]. The WHO also stated that a true definition of biomarkers includes "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, and biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction" [4]. Examples of biomarkers include everything from pulse and blood pressure through basic chemistries to more complex laboratory tests of blood and other tissues [5,6].

Disease-related biomarkers give an indication of whether there is a threat of disease (risk indicator or predictive biomarkers) if a disease already exists (diagnostic biomarkers), or how such a disease may develop in an individual case (prognostic biomarker). Drug-related biomarkers indicate whether a drug will be effective in a specific patient and how the patient's body will process it.

Biomarkers a measure of a normal biological process in the body, a pathological process, or the response of the body to therapy – may offer information about the mechanism of action of the drug, its efficacy, its safety, and metabolite profile. Because biomarkers can predict drug efficacy more quickly than conventional clinical end-points, they hold the potential to substantially accelerate product development in certain disease areas. And because they help to identify earlier those candidates that are likely to fail, they reduce drug development costs, giving life to the concept of "fail early, fail cheap."

Biomarkers have impacted on internal decision-making, i.e., whether to move forward to the next phase of clinical development or not. The decision to move to next phase depends not only on biomarker evidence alone but also they can offer strong supporting evidence, and in the future, it will be the key data in certain programs and offers an objective, biological indicator, rather than just seeing whether the patients feel better. In the present scenario, there is no possibility of developing a new drug without simultaneously looking for biomarkers for efficacy, safety, and to measure the pharmacodynamics of the drug. Mechanistic or target biomarkers can be used in the pre-clinical or phase I trials to measure the pharmacological effect of the drug, i.e., whether the drug interacts with its receptor, enzyme, or protein target, whether it is distributed to the site where it needs to act, whether there is some

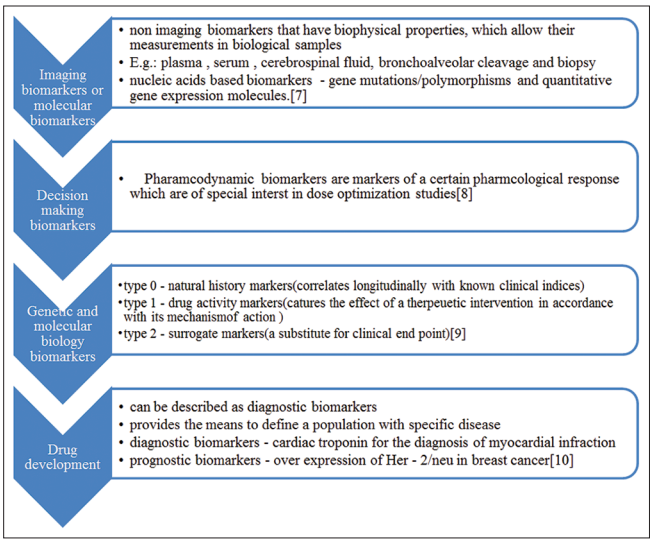

Fig. 1: Classification of biomarkers

form of downstream pharmacology, and the dose ranges, in which the drug is pharmacologically active. Hence, these types of biomarkers can be used to drive critical go/no – go decisions in drug development (Tables 1 and 2, Figs. 1-4).

At the onset of cancer, a selective protein or gene-based biomarker gets elevated or modified in body fluids or tissues. Early diagnosis of these markers can greatly improve the survival rate or facilitate effective treatment with different modalities. Although the

Table 1: Techniques available for biomarker development

Technology	Method	Objective	Tissue
Genomics	SNP genotyping, positional cloning/microsatellites, expression analyses	Identify susceptibility or disease-modifying gene Fine mapping/sequencing of disease loci Identification of different expression of genes and signaling pathways	Nucleated cells, diseased tissue
Proteomics	2DGE, MS, LC-MS, GC-MS, MS-MS, MALDI–TOF MS	Identification of low – abundance proteins, their subcellular location, post-translational modification, interactions among proteins	Urine, blood, saliva, tissues
Metabolomics	NMR spectroscopy, MS, infrared spectroscopy	Small molecule identification and characterization	As above
Pharmacogenetics	SNP genotyping	Relate genetic makeup to drug response	Nucleated cells
Integratomics	All of the above	Use of high throughput technology to produce an integrated picture at the DNA, RNA, protein, tissue, and pharmacological levels	All of the above
Bioinformatics	BLAST, hierarchical clustering, SOM	Link microarray data to biological pathways	Data from various techniques
Molecular imaging [13]	CT, MRI, PET, SPECT, biophotonic imaging	Noninvasively identify and quantify the causative molecular constituents of diseased tissues in time and space	Patients

MRI: Magnetic resonance imaging, CT: Computed tomography, PET: Positron emission tomography, SPECT: Single-photon emission computed tomography, SNP: Single nucleotide polymorphism, MS: Mass spectroscopy, NMR: Nuclear magnetic resonance, 2DGE: 2D gel electrophoresis, TOF: Time-of-flight, LC-MS: Liquid chromatograph-mass spectroscopy, GC-MS: Gas chromatography-mass spectrometry, SOM: Self-organizing maps

Table 2: Five phases of biomarker development

Phases [14-16]	Phase 1 Preclinical exploratory	Phase 2 Clinical characterization and assay validation	Phase 3 Clinical association: Retrospective screening studies	Phase 4 Clinical association: Retrospective screening studies	Phase 5 disease control
Objective	Target biomarker identification, feasibility	Study assay in people with and without disease	Case-control studies using respiratory specimens	Longitudinal studies to predict disease	Clinical use
Site	Biomarker development laboratory	Biomarker validation laboratory	Clinical, epidemiologic centers	Cohort studies	Community
Design	Cross-sectional	Cross-sectional	Case-control	Prospective	RCT
Sample size	Small	Small	Modest	Medium	Large
Validity	Content and construct validity	Criterion validity	Predictive validity	Efficacy of strategy	Effectiveness
Result	Assay, precision, reliability, sensitivity	Reference limits, intra-individual variation	Screening characteristics, true and false+rates	ROC analyses	No needed to screen treat

RCT: Randomized controlled trial, ROC: Receiver operating characteristic

Table 3: Breaking the barriers to biomarker discovery

Barrier to cancer biomarker progress [17,18]	Emerging successful strategies to break the barrier
Failure to mechanistically tie a blood biomarker to the tumor itself	Discovery of the biomarker across a series of experimental animal tumor models Mechanistically showing a role in tumor genesis or a change after therapy Validation of the same marker using human samples
Improper sample handling and tracking; inadequate tissue fixation and body fluid sample preservation that generates bias, false positives, and false negatives	Preservation technologies for tissue and body fluid sample collection Uniform protocols for the collection of tissues and body fluids Molecular measures to verify the reservation of a biological sample
Lack of independent blinded clinical validation with proper controls for specificity and noncancer diseases	Inclusion of independent epidemiologically credentialed and matched cohorts with inflammatory disease, infectious disease, and benign tumors
Low analytical sensitivity of mass spectrometry-based detection systems that prevent the detection/identification and measurement of low abundance (<ng/nl) biomarkers emanating from early stage cancer	Nanotechnology-based methods for biomarker capture, preservation, and exclusion of unwanted high-abundance proteins such as albumin can amplify mass spectrometry sensitivity 1000

sophisticated imaging technologies such as magnetic resonance imaging, positron emission tomography, and computed tomography have the impact of nanotechnology on their improved performance, they are however unsuitable for the early detection of cancer biomarkers or their quantification. Other approaches for cancer diagnosis based on cell morphology and microscopy (biopsies) are too not conclusive for early diagnosis of cancer. The only hope for early diagnosis of cancer in the near future is by the detection of cancer biomarkers using immunoassays/sensors that are reformed by Nanotechnology. Attractive properties of nanoparticles have miraculously lifted up the design, fabrication, and sensitivity and multiplexing of these immunoassays/sensors in biomarker detection (Tables 3-7).

CONCLUSION

Biomarkers are biological molecules wit physiological characteristics that are more closely linked to the underlying causes of health or disease. Doctors customarily answer the questions

- Is a patient really sick?
- What medicine is necessary?
- In what dosage?
- Is the patient responding to it?

Based on a variety of symptoms which are giving subjective description and uncertain relationship to the disease state are misleading. Biomarkers give doctors a more objective and quantifiable basis for clinical decision-making.

Table 4: Biomarker of cancer disease and their characteristics with examples

Cancer	Markers	Characteristics	Typical sample
Prostate	PSA, total and free	High sensitivity in all stages; also elevated from some non-cancer causes	Blood [19]
	PSMA	Levels tend to increase with age	Blood
Breast	CA 15-3, 27, 29	Elevated in benign breast conditions. Either CA 15-3 or CA 27, 29 could be used as marker	Blood [20,21]
	Estrogen receptors	Overexpressed in hormone-dependent cancer	Tissue [22]
	Progesterone receptors		Tissue
	Her-2/neu	Only 20~30% of patients are positive to Her-2 oncogene that is present in multiple copies	Tissue [23]
Lung (non-small cell)	CEA	Used in combination with NSA to increase specificity, used also for colon cancer detection	Blood [24]
Lung (small cell)	NSE	Better sensitivity toward specific types of lung Caner	Blood [25]
Bladder	NMP-22, BTA	NMP-22 assays tend to have greater sensitivity than BTA assays	Urine [26]
Pancreatic	BTA	Composed of basement membrane complexes	Urine [27]
	CA 19-9	Elevated also in inflammatory bowel disease, sometimes used as colorectal cancer biomarker	Blood [28]
Epithelial ovarian cancer (90% of all ovarian cancer)	CA 125	High sensitivity in advanced stage; also elevated with endometriosis, some other diseases and benign conditions	Blood [29]
Germ cell cancer of ovaries	CA 72-4	No evidence that this biomarker is better than CA-125 but may be useful when used in combination	Blood [30]
	AFP	Also elevated during pregnancy and liver cancer	Blood [31]
Multiple myeloma and lymphomas	B2M	Present in many other conditions, including prostate cancer and renal cell carcinoma	Blood [32]
	Monoclonal immunoglobulins	Overproduction of an immunoglobulin or antibody, usually detected by protein electrophoresis	Blood, Urine [33]
Metastatic melanoma	S100B	Subunit of the S100 protein family	Serum [34]
	TA-90	Could be used to monitor patients with high risks of developing the disease	Serum [35]
Thyroid	Thyroglobulin	Principal iodoprotein of the thyroid gland	Serum, Tissue [36]
Thyroid medullary carcinoma	Calcitonin	Secreted mainly by parafollicular C cells	Blood, Serum [37]
Testicular	hCG	May regulate vascular neoformation through VEGF	Serum [38]
WM	Monoclonal immunoglobulin M	The larger size and increased concentration of the monoclonal protein leads to serum hyperviscosity, the most distinguishing feature of WM	Blood, Urine [39]
Lymphomas	B2M	Present in many other conditions, including prostate cancer and renal cell carcinoma	Serum [40]
Lung (non small cell), epithelial, colorectal, head and neck, pancreatic, or breast	EGFR (Her-1)	Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation	Tissue [41]
Colorectal, lung, breast, pancreatic, and bladder	CEA	Subtle posttranslational modifications might create differences between tumor CEA and normal CEA	Serum [42]
T-ALL	PTK7	Membrane-bound surface protein of whole cells, and can be used to detect circulating tumor cells as targets	Blood [43]

PSA: Prostate-specific antigen, PSMA: Prostate-specific membrane antigen, CA 15-3, 27, 29: Cancer antigen 15-3, 27, 29, CEA: Carcinoembryonic antigen, NSE: Neuron-specific enolase, NMP: Matritech's nuclear matrix protein, BTA: Bladder tumor antigen, CA 19-9: Carbohydrate antigen 19-9, CA 125: Cancer antigen 125, CA 72-4: Cancer antigen 72-4, AFP: Alpha-fetoprotein, B2M: Beta-2 microglobulin, TA-90: Tumor-associated glycoprotein antigen, hCG: Human chorionic gonadotropin, VEGF: Vascular endothelial growth factor, WM: Waldenstrom's macroglobulinemia, T-ALL: T-cell acute lymphoblastic leukemia, EGFR: Epidermal growth factor receptor

Table 5: Cardiovascular diseases

Name of disease	Effects	Risk score (%)	Biomarker
Homozygous familial hypercholesterolemia	Premature cardiovascular morbidity and mortality	10-20	TC, LDL cholesterol [44]
Hypertriglyceridemia/hypertriglyceridemia	Elevated levels of Lp(a)	20	Lipid profile [45]
Chronic kidney disease	Elevated levels of Lp(a)	10	Lipid profile [46]
Cholelithiasis	Gallstone formation due to cholesterol and salts	20	LDL cholesterol and small dense LDL particles [47]
Hypercholesterolemia	Very high CVD risks	20	LDL cholesterol and small dense LDL particles [48]
Atherosclerosis	Arterial obstruction, chest pain	20-25 ABCA1 Efflux	PUFA and carbohydrates, serum γ-glutamyl transferase activity, blood genomic profiling, and α4β7 integrin [49]
Coronary heart disease	Monocytosis, high diabetics, hypertension, and chronic kidney diseases	20	Impaired sterol efflux, efflux capacity of HDL, myeloperoxidase increasing circulating HDL [50]
Hyperglycemia or type 1 diabetes	CVD and mortality	25	TC, TG, HDL, LDL, and anthropometric and biochemical parameters [51]
Dyslipidemia	Hypoperfusion, high inflammation, and low BP	10	TC, TG, HDL, LDL, and anthropometric and biochemical parameters [52]
Atherosclerotic peripheral arterial disease	Prevalent, morbid, and mortal diseases	20 shortening of lumen	LDL cholesterol [53]
IHD	Endothelial dysfunction, vascular inflammation	10-20	Lipids, cholesterol, calcium, and cellular debris [54]
Diastolic dysfunction and diastolic heart failure	Asymptomatic hypertension	20	Myocardial remodeling [55]
Chronic heart failures	ADP-induced platelet aggregation, triglycerides, end-diastolic volume, end-diastolic dimension, and ventricular sepal thickness death	15-20	Lipidemic, hemostasiological, and hemodynamic indicators, Willebrand factor, and D-dimer [56]
Myocardial infarction	Very high morbidity, severe pain	20-25	Circulating microRNAs level in patients [57]
Lipid stress and storage	Influence cholesterol availability in lipid rafts in immune cells	High LDL/HDL cholesterol levels	Omega-3 index [58]
Neuronal dysfunction	Neuronal cell death and neuroinflammatory	10-15	27-hydroxycholesterol, plasma HDL, NAEs [59]
Transient global cerebral ischemia	Cardiac arrest and cardiovascular Problems	5-10	ω-3 PUFAs [60]
Hypoglycemia	Cardiac implications	5-10	Elevated levels of Lp(a) and low HDL cholesterol [61]
Hypertriglyceridemia/CAD/acute coronary syndrome	Severe effect on BMR and peripheral and cardiac circulation	5-10	Altered serum lipid [62]
HDL metabolism disorders	Severe inflammation and pain	5-10	LDs [63]
Nephrotic syndrome	Renal filtration chocked	5-10	LDL cholesterol, triglycerides, and Lp(a) [64]
Fatal myocardial infarction and brain stroke	Cardiovascular risks, morbidity, and mortality in elderly men	20-25	Fat-specific protein Fsp27, FIT proteins, seipin, and ADP-ribosylation factor 1-coat protein complex I [65]
Systemic lupus erythematosus	Problem of PCV and hemoglobin	5	Factors, proteins, ions, and stimulators of heart muscles [66]
Acute myocardial infarction	Death of part of myocardial muscles, central chest pain, and severe crushing	20-25	Serum soluble ST2 and interleukin-33 [67]
Hypertension and dyslipidemia, hypercholesterolemia	Cardiovascular risk factors	15-20	TC and LDL [68]
		5-10	BP and LDL-C, high BMI [69]
SCVRs	Tachyarrhythmias, bradyarrhythmias	5	LDL-C, HDL-C, TG, ApoAI, and ApoB Lp(a) [70]
AVDs, type 2 diabetes, or metabolic syndrome	Increased levels of triglycerides, low levels of high-density lipoprotein cholesterol, and postprandial lipemia	20-25	MetS [71]
Procardiovascular risks, cardiovascular risks	Inflammation, obesity, and thrombosis	5-10	Sedentary behavior, β-trace protein from GFR marker [71]

(*Contd*...)

Table 5: (*Continued*)

Name of disease	Effects	Risk score (%)	Biomarker
Metabolic lipid disorders	Circulatory dysfunctions, high BP, peripheral pain, and high or low BMR	5-10	MALDI-MS, imaging, and lipidomics for clinical diagnosis, and proteome analysis [71]
Ischemic heart disease	Circulatory dysfunctions	Smoking, hypertension, age, family history	Endothelial dysfunction, monocyte accumulation, endothelial apoptosis, and thrombus formation [71]
Low HDL-C syndromes	Increased risk of CAD	5	Sphingomyelin phosphodiesterase 1 and glucocerebrosidase [71]
Hypothyroidism and gall stone	Severe pain, inflammation	5	TSH level and sodium and potassium salts [71]
Multiple CVDs, diabetes, stroke, and recurrent ischemia syndrome	Hepatic inflammation due to common carotid intima-media thickness	10-20	Multiple biomarkers, vascular imaging [71]
Angina pectoris	Obesity, arterial thickness, BMI, and respiration rate, and severe chest pain	10-0	Coronary angiography [71]
Antiphospholipid syndrome	Venous thrombosis	5	microRNAs [71]
Myocardial infarction	PAPP-A in serum	15	Severe blood pressure changes, central chest pain, and silent or knocking angina [71]

HDL: High-density lipoprotein, TC: Total cholesterol, LDL: Low-density lipoprotein, CVD: Cardiovascular disease, PUFA: Polyunsaturated fat, IHD: Ischemic heart disease, TG: Triglyceride, NAEs: N-acylethanolamines, LDs: Lipid droplets, CAD: Coronary artery disease, FIT: Fat storage-inducing transmembrane, BMI: Body mass index, PAPP-A: Pregnancy-associated plasma protein-A, TSH: Thyroid-stimulating hormone, BMR: Basal metabolic rate, BP: Blood pressure, GFR: Glomerular filtration rate, AVD: Atherosclerotic vascular disease, SCVRs: Spinal cord vascular resistances, MS: Mass spectroscopy, MetS: Metabolic syndrome

Table 6: Hepatocellular carcinoma biomarkers

HCC marker	Clinical use
AFP	Early diagnosis, monitoring, and recurrence
Lens culinaris agglutinin reactive AFP (AFP-L3%)	Early diagnosis and prognosis, vascular invasion
DCP	Early diagnosis and prognosis, portal vein invasion and metastasis
Gamma-glutamyl transferase	Early diagnosis complementary to other markers
Alpha-l-fucosidase	Early diagnosis
Glypican-3	Early diagnosis
Human carbonyl reductase 2	Prognosis
Golgi phosphoprotein 2	Tumor aggressiveness
Transforming growth factor beta	Tumor invasiveness
HGF	Prognosis and disease recurrence
TGF-b	Prognosis invasiveness
Tumor-specific growth factor	Diagnosis complementary to other markers
Epidermal growth factor receptor family	Early recurrence
Hepatocyte growth factor	Metastasis reduced survival
Micro RNAs	Tumor spread and survival [72]

AFP: Alpha-fetoprotein, DCP: Des-gamma-carboxy prothrombin, HGF: Hepatocyte growth factor, TGF-b: Transforming growth factor-b, HCC: Hepatocellular carcinoma

Table 7: Analytical method to discover biomarker for Alzheimer's disease diagnosis

Analytical method	Biomarker
ELISA	Aβ42, total tau, phospho – tau – 181 (single)
Multiplex searchlight ELISAs	16 signaling proteins
Filter-based array sandwich ELISA	18 signaling proteins
INNO - BIA AlzBio3 Luminex – based technology (innogenetics)	Aβ42, total tau, phospho – tau – 181 (multiplex)
Tissue array	2325 tissue specimens
Quantitative real-time RT-PCR	33 genes, multiple phosphorylated tau epitopes
Liquid chromatography/electrosprayy ionosation MS	Aβ 40, Aβ42
Capillary electrophoresis/MS	1000 polypeptides
Ultrasensitive laser ablation inductively coupled plasma/MS	Trace elements and metal ions
Multiplex iTRAQ	1500 CSF proteins
Surface-enhanced laser desorption/ionization or matrix-assisted laser desorption/ionization	Several Aβ species: Aβ37, Aβ36, Aβ38, Aβ40
DNA/RNA chips, biochips, gene chips	Several thousand genes [73]

MS: Mass spectroscopy, CSF: Cerebrospinal fluid, RT-PCR: Real-time polymerase chain reaction

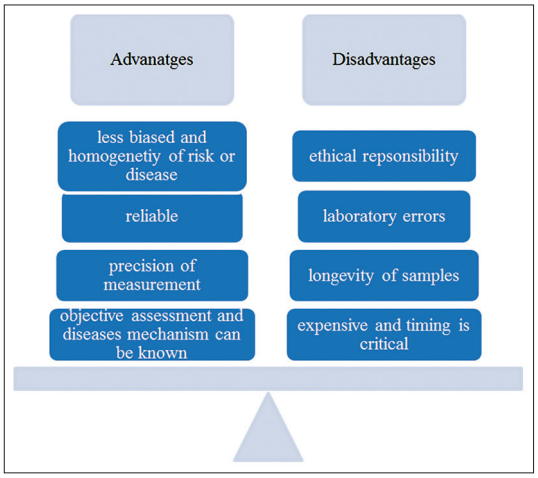

Fig. 2: Relative advantages and disadvantages of biomarkers [11]

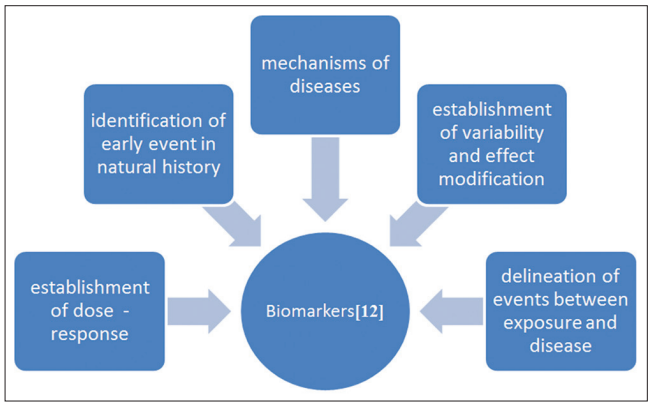

Fig. 3: Salient features of biomarkers

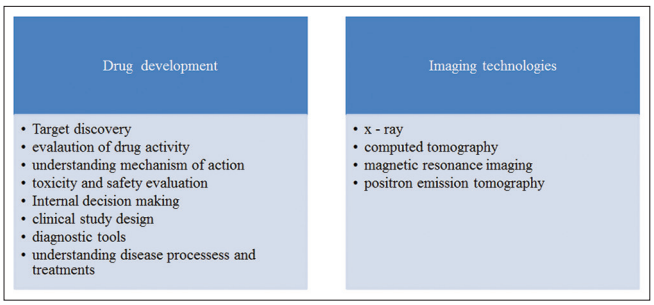

Fig. 4: Biomarkers applications

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