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ROLE OF BIOMARKERS IN CARDIOTOXICITY

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ABSTRACT

Cardiotoxicity is the 'toxicity that affects the heart which includes direct effect of the drug on the heart as well as indirect effect due to enhancement of haemodynamic flow alterations or due to thrombotic events. It is associated with small changes in blood pressure and arrhythmias to cardiomyopathy. Hypertrophic cardiomyopathy (HCM), a cardiac disorder is associated with asymmetric cardiac hypertrophy with involvement of the interventricular septum. HCM is caused by mutation in genes such as Myosin-binding protein C ($MYBC_3$), heavy chain of beta myosin (MYH_7), troponin T ($TNNT_2$) and troponin I ($TNNI_3$). The most recurrent mutation is seen in $MYBC_3$, MYH_7 around 13-25% while $TNNT_2$, $TNNI_3$ shows below 5%. Similarly, pulmonary arterial hypertension (PAH) is an obstructive disorder of the smallest pulmonary arteries, in which progressive narrowing increases pulmonary vascular resistance which leads to right heart failure and death. PAH is caused by imbalance of activation of transforming growth factor--β (TGF-β) receptors coupled with the impaired mutations in bone morphogenetic proteins receptor type 2 ($BMPR_2$) which involves bone growth and repair. Therefore, cardiotoxicity can be assessed by markers such as Troponin T which is indicative for myocardiocyte damage and is used in the diagnosis of myocardial ischemia. Anthracycline is a cytotoxic drug which causes elevation of Troponin T level which is associated with a decrease in left ventricular ejection fraction. Another biochemical marker is BNP (B type-natriuretic peptide), a hormone secreted by the myocytes of the heart. Elevation in the level of BNP causes increase in plasma concentrations which lead to left ventricular dysfunction.

Key words: Hypertrophic cardiomyopathy, B type-natriuretic peptide, Myosin-binding protein C, Heavy chain of beta myosin, Troponin T, Troponin I, , Bone morphogenetic proteins receptor type 2.

Cardiotoxicity is the 'toxicity that affects the heart' which includes a direct effect of the drug on the heart but also an indirect effect due to enhancement of haemodynamic flow alterations or due to thrombotic events [1]. Cardiotoxicity may be caused by chemotherapy treatment, complications from anorexia nervosa, adverse effects of heavy metals intake, or an incorrectly administered drug such as bupivacaine [2]. The mechanism of cardiotoxicity leads to over expression of the free radicals and consequently leading to oxidative stress, with apoptosis of cardiac cells or immunologic reactions, A minor loss of left ventricle pumping efficiency causes shortness of breath. It may also result in congestive heart failure (CHF), heart attack, or death [3]. Cardiotoxicity includes a wide range of cardiac effects from small changes in blood pressure and arrhythmias to cardiomyopathy (Fig 1).

Mutation in genes causes various disorders

Cardiomyopathy is a progressive deterioration of heart muscles (myocardium), common

symptoms are dyspnea (breathlessness) and peripheral edema (swelling of the legs) [5]. Cardiomyopathy, with an estimated prevalence of between 1:500 and 1:5000, is characterized by impaired contractile function of the myocardium, in some cases results into arrhythmias, embolic stroke and sudden cardiac death. Myocardial dysfunction linked with cardiomyopathy can either be mechanical or electrical and sometimes may not include structural abnormalities [6].

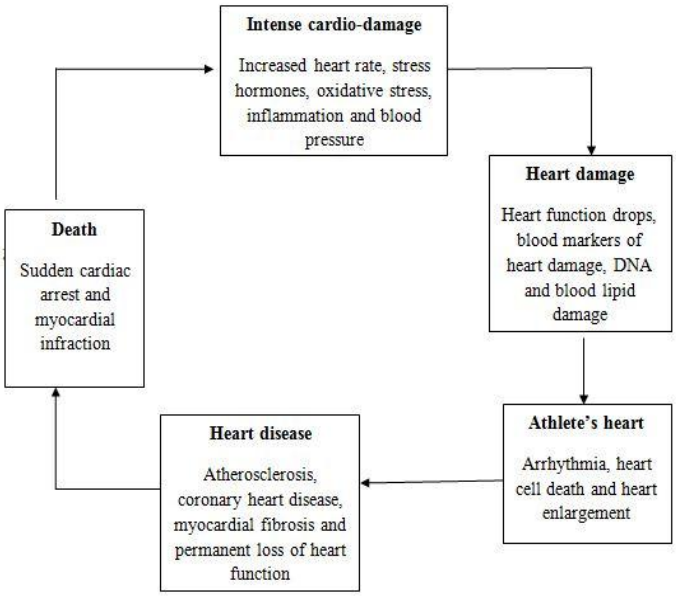

Figure 1: Cycle of cardiotoxicity [4]

The four most common forms of cardiomyopathy are hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C), and left ventricular noncompaction (LVNC).Rarer forms of cardiomyopathy include restrictive cardiomyopathy (RCM) and the amyloid-associated cardiomyopathies, including transthyretin amyloidosis (ATTR) and apoliprotein A1 amyloidosis (AApoA1) [7]. Cardiomyopathy is caused by mutations in genes coding for structural or regulatory proteins associated with heart muscle sarcomere [8]. The genes responsible for cardiomyopathy are heavy chain of beta-myosine (MYH7) were mostly characterised by moderate to severe hypertrophy of the left ventricle. While mutations of the Troponin T (TNNT2) [9] gene were characterised by minimal left ventricular hypertrophy. Myosin binding protein (MYBPC3) mutations are associated with slower development of left ventricular hypertrophy [10]. The most recurrent mutation is seen in MYBC3, MYH7 around 13-25% [11] while TNNT2, TNNI3 shows below 5% [12]. Furthermore, Pulmonary Arterial Hypertension (PAH) is characterized by abnormal pulmonary vascular remodeling, endothelial vasoconstriction, and thrombosis in-situ, ultimately leading to elevated pulmonary vascular resistance (PVR) which leads to right heart failure and death [13]. It is an enigmatic and fatal disease that affects predominantly women (female–male ratio, 3:1) with symptoms like dyspnea on modest exertion, loss of energy and signs of right ventricular strain. Idiopathic PAH affects the smallest pulmonary arteries of the lung. Intimal proliferation and fibrosis occlude the lumen, whereas muscular hypertrophy of the media also contributes to increased resistance to flow shown in Fig 2 [14]. PAH is associated with mutations in genes such as TGF-β receptor signals that affect vascular intimal proliferation. The TGF-β super family consists of cytokines and their receptors that control the sequence of cell and tissue growth which stimulate wound healing and inflammatory responses and leads to neoplastic transformation of cells [16,17]. The TGF system is named as super family because it comprises of more than 40 similar protein cytokines (TGFs or bone morphogenetic proteins [BMPs]) that activate serine–threonine cell surface receptors which transfer the signal through intracellular pathways by phosphorylating Smad proteins. Smads are intracellular signalling proteins first found in drosophila and roundworms which move from the cytosol to the nucleus and regulate specialized proteins called nuclear transcription factors that activate or repress transcription of DNA. Another, genes associated with PAH is BMP receptor type 2 (BMPR2) which are involved in bone growth and repair [18,19]. Bone morphogenetic receptor type 2 is activated primarily by the cytokines BMP 2, 4, and 7 and does not respond to TGF-β. More than 140 BMPR2 mutations have been identified in patients with heritable PAH [20, 21]. BMPR2 mutations cause a loss of receptor function, either through missense (wrong amino acid), nonsense (stops transcription of DNA into RNA at the site of the mutation onward) or frame shift alterations of the codon. Similarly, Atherosclerosis is an inflammatory disease which causes deposition of cholesterol and inflammatory cells in the arterial wall [22]. In the

later stages, thrombosis occurs which leads to myocardial and cerebral infarctions [23,24].

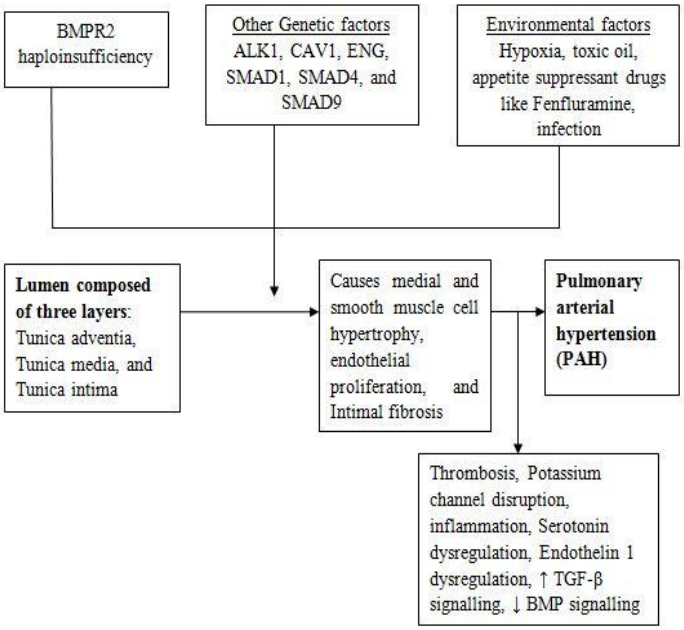

Figure 2: Genetic and environmental factors involved in the aetiology of PAH. [15] (Where TGF- β, transforming growth factor-β; BMP, bone morphogenetic proteins; BMPR2, bone morphogenetic proteins receptor type 2; ALK1, activin receptor-like kinase 1; CAV1, Caveolin-1; ENG, endoglin)

Recently, it was studied that atherosclerosis is caused by mutation in genes such as ryanodine receptor gene (RYR3) gene which is located on chromosome 15q14-q15 which encodes a large intracellular homotetrameric protein (> 2MDa) that comprises 4780 amino acids [25-27]; RYR3 protein resides on the sarcoplasmic reticulum membrane and releases Ca^{2+} from intracellular stores to regulate intracellular calcium concentration [28,29]. In human arterial endothelial cells, Ca^{2+} release is mediated by RYR3, but not by the RYR1 and RYR2 which play a role in endothelial vasodilation [30]. RYR3 have a major role in calcium signalling in the vasculation and thus it plays a major role in the pathogenesis of atherosclerosis. Along with the function of RYR3 in vascular cells, it is also shown to possess function in T cells, which is involved in the inflammation process of atherosclerotic lesions [31, 32]. RYR3 appear to regulate Ca^{2+} signalling in T lymphocytes, in which, calcium signalling is essential for its activation and differentiation. The association of T cell activation markers and carotid arterial stiffness was also reported in a group of HIV-infected women [33].

The study suggested that, intracellular calcium mobilization mediated by RYR3 is involved in atherosclerosis process through participation of arterial smooth muscle cells, endothelial cells and T lymphocytes. This increase in cardiotoxicity which arises due to mutation in genes can be easily assessed by various biomarkers.

ROLE OF BIOMARKERS IN ASSESSMENT OF CARDIOTOXICITY

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality in the United States [34]. Biomarkers are an important tool to better identify high-risk individuals, to diagnose disease conditions promptly and accurately, and to effectively prognosticate and treat patients with disease. The term Biomarkers (biological markers) means "measurable and quantifiable biological parameters which include specific enzyme concentration, hormone concentration, specific gene phenotype distribution in a population, presence of biological substances which serve as index for health and physiology-related assessments, such as psychiatric disorders, environmental exposure and its effects, metabolic processes, substance abuse, pregnancy, cell line development, epidemiologic studies". In 2001, NIH working group harmonized the explanation of a biomarker as "a characteristic that is impartially measured and assessed as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention". There are various types of Biomarkers which have been listed below:

Biomarkers: A characteristic that is impartially measured and assessed as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention.

- **Type 0 Biomarkers**: A marker of the natural history of a disease and correlates longitudinally with known clinical indices
- **Type 1 Biomarkers**: A marker that captures the effects of a therapeutic intervention in accordance with its mechanism of action.
- **Type 2 biomarker** (**Surrogate end point):** A marker that is intended to substitute for a

clinical end point; a surrogate end point is expected to predict clinical benefit (or harm or lack of benefit or harm) on the basis of epidemiological, therapeutic, patho-physiological, or other scientific evidence.

- **Risk factor**: A risk factor is associated with a disease because it is in the causal pathway leading to the disease.
- **Risk marker**: A risk marker is associated with the disease (statistically) but need not be causally linked; it may be a measure of the disease process itself.
- **Clinical end point**: A characteristic or variable that reflects how a patient feels, functions, or survives.
- **Intermediate (non-ultimate) end point**: A true clinical end point (a symptom or measure of function, such as symptoms of angina frequency or exercise tolerance) but not the ultimate end point of the disease, such as survival or the rate of other and irreversible morbid events.
- **Validation of a biomarker (assay or method validation)**: A process for assessing performance characteristics (i.e., sensitivity, specificity, and reproducibility) of a biomarker measurement or an assay technique.
- **Qualification of a biomarker (clinical validation)**: The evidentiary process linking a biomarker to disease biology or clinical outcome.
- **Evaluation of a biomarker**: A process of linking biomarkers to outcomes, often with a view to establish surrogate status.

Moving ahead of a sole focus on Myocadial infraction (MI), the search for alternative and supplementary serum markers to assist in sorting out the presence, severity and type of cardiac injury have been shown in Fig 3.

RECENT ADVANCES IN BIOMARKERS

A number of additional novel clinical markers have also been studied recently. Some of these show promising results as early prognostic and diagnostic markers.

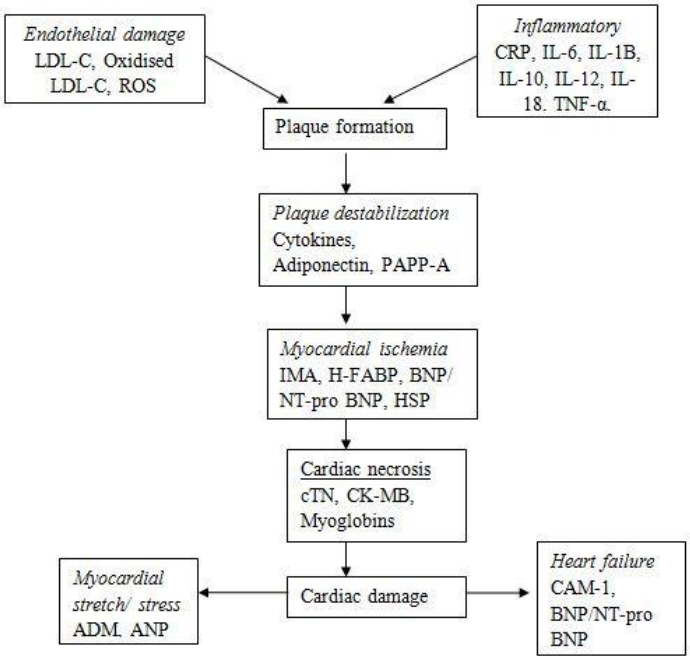

Figure 3: Development of Cardiac markers (Where ADM, adrenomedullin; BNP, B-type natriuretic peptide; CAM, cell adhesion molecule; CK-MB, creatine kinase-MB; CRP, C-reactive protein; cTn, cardiac troponin; H-FABP, human fatty-acid binding protein; HSP, heat shock protein; IL, interleukin; IMA, ischemia-modified albumin; PAPP, pregnancy-associated plasma protein; ROS, reactive oxygen species [36].)

From the past 10 years, asymmetric dimethylarginine (ADMA) received much attention as a promising cardiovascular biomarker. ADMA is an endogenous competitive inhibitor of nitric oxide synthase which can also cause vasoconstriction [37]. Its level is found to be increased in various cardiovascular events like atherosclerosis, hypertension, coronary artery disease and chronic heart failure. ADMA has also been associated with inflammation and increased risk of death in cardiovascular related events [38]. Myeloperoxidase (MPO) is another recently described biomarker that has been found to be important for heart failure, acute coronary syndrome and, recently, atherosclerosis [39,40]. MPO is an enzyme which can produce hypochlorite and which is released during the early phase of inflammatory process and has been linked to both inflammation and oxidative stress. MPO has been related to CVD due to its involvement in LDL and HDL oxidation which is closely related to plaque formation in arterial walls through increased cholesterol aggregation. MPO shows an early promising result which is able to demonstrate diagnostic value of CVD which are showing negative results for Troponin T. F2 isoprostanes are a family of prostaglandin compounds derived

from arachidonic acid peroxidation which have recently shown promising potential as in vivo markers of oxidant injury in cardiovascular pathologies such as atherosclerosis, hypertension and recently in Acute coronary syndrome (ACS) [41,42, 43]. Even though increased levels of this marker have been found in non-cardiovascular related pathologies such as Alzheimer's disease, pulmonary disorders and renal failure, its presence has been strongly linked with well-known cardiovascular risk factors [44]. Despite these promising results, F2 isoprostanes have not been used on a large scale. Caveolae are flask-like invaginations [45] that create signalling microdomains of the plasma membrane enriched with cholesterol, sphingolipids, the marker protein caveolin, and the coat protein cavin [46,47]. Caveolins have three isoforms (caveolin 1-3) and cavins consist of four isoforms (cavin 1-4). Caveolin-3 [48] and cavin-4 [49] are expressed predominantly in cardiac muscle and have been identified as important proteins involved in cardiomyopathy [50,51]. In cardiomyocytes, there are many different signalling molecules that are concentrated and organized within the caveolae, and these can mediate signal transduction. Recent studies suggest that caveolae and caveolae-associated signalling molecules play an important role in protecting the myocardium against I / R injury

Clinically relevant cardiac markers

Cardiac troponins

The troponin protein complex consists of 3 subunits, the C (TnC) subunit which is the calcium binding component, the I (TnI) which maintains the structural position of the troponin-tropomyosin complex, and the T (TnT) which is the tropomyosin binding subunit [52]. These are located on the thin filament of both skeletal and myocardial myocytes, the latter playing an integral role in the Frank-Starling mechanism of the heart [53, 54]. Interestingly, both TnT and TnI subunits have distinct isoforms for each muscle type, hence there is a specific cardiac isoform [55]. Cardiac troponins T and I (cTnT and cTnI) are now recognized as the most tissue-specific biomarkers related to cardiac damage and have been included as a diagnostic criterion for several cardiac-related pathologies. This success is closely related to the troponins' unique position and function in the cardiomyocyte and the ability to generate specific monoclonal antibodies against both cTnT and cTnI which are precise tissue-specific biomarkers of myocardial injury that are not detected in healthy individuals [53].

Osteopontin (OPN)

Osteopontin (OPN) is a matricellular glycoprotein/ cytokine that has been recently found to be a promising prognostic biomarker for patients with heart failure, ischemic heart disease and cardiac remodelling in both clinical and pre-clinical settings [56-59].OPN has been described as a regulator of inflammation and bio-mineralisation via macrophage interaction while associated with bone remodelling. OPN has been characterised as an independent predictor of death within 4 years for patients with heart failure and was found highly elevated in patients with left ventricular dysfunction. However, OPN is expressed in many tissues and has been described as a marker in non-CVD related pathologies like cancer, myeloma, multiple sclerosis, bone destruction, angiogenesis, Graves' disease and pulmonary hypertension.

Brain natriuretic peptide (BNP)

Measurement of plasma brain natriuretic peptide (BNP) concentration is a very efficient and cost- effective mass screening technique for identifying patients with various cardiac abnormalities, regardless of aetiology [60]. BNP is a 32-amino acid polypeptide cardiac neuro-hormone secreted from membrane granules in the cardiac ventricles, particularly the left ventricle, as a response to ventricular volume expansion and pressure overload [61]. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (B-NP) are of myocardial cell origin, while C-type natriuretic peptide (CNP) is of endothelial origin [60]. BNP was originally named brain natriuretic peptide and was first detected in porcine brain. BNP levels have been found elevated in patients with various clinical conditions such as heart failure, Myocardial infraction (MI), left ventricular hypertrophy, cardiac inflammation, primary pulmonary hypertension, renal failure, ascetic cirrhosis which is associated with advanced age. The levels correlate with severity of symptoms as well as with prognosis which helps to detect the presence of heart failure, determine its severity

and estimate prognosis. BNP has the potential to improve the management of patients with congestive heart failure (CHF) and may become a routinely assessed serum parameter in clinical medicine. BNP is less costly than other tests for CHD, and due to its cost-effectiveness is highly desirable in developing countries. Recently, NT-proBNP was approved by the FDA for use in assessing the prognosis of patients with congestive heart failure and acute coronary syndrome, while the BNP assay is also approved for risk stratification in acute coronary syndrome.

Creatinine kinase

Creatinine kinase MB is an enzyme present primarily in cardiac muscle. The MB is one of the three CK isoenzymes the other being the MM and BB. CK-MB is released rapidly after myocardial injury [62]. During an onset of acute myocardial infarction (AMI), CK-MB rises to twice the normal levels within 6 hours and peaks within 12–24 hours. CK-MB plays an important role in defining the infarct size, expansion and risk of re-infarction. If a cTn is not available, the CK-MB is considered the best alternative marker of AMI. Decades ago, elevated serum levels of CK-MB, the cardiac-specific isoform of CK, were also used as biomarkers for the diagnosis of myocardial necrosis [63]. Even though the CK-MB has been proven a relatively sensitive measure of myocardial necrosis and AMI, this enzyme is not exclusively specific to myocardial damage, as elevated levels in several conditions following acute or chronic muscle injury and in patients undergoing surgical procedures, have been found [64]. Furthermore, CK is present in the intestine, diaphragm, uterus and prostate, and injury to these organs would result in release of CK-MB and thus impair the specificity of CK-MB serum measurements.There is no clear consensus on whether absolute CK-MB or the CK-MB relative index is the preferred test for patients with potential acute coronary syndromes, but the World Health Organization international diagnostic criteria and several others recommend use of absolute CK-MB [65].

DIAGNOSIS

Over the years, several imaging modalities have been developed that vary significantly in precision, ease of use, availability and costs [66]. LVEF may be measured by planar multigated radionuclide angiography (MUGA), quantitative gated blood-pool SPECT (GBPS), 2- and 3-dimensional echocardiography, radiographic contrast angiography or cardiac MRI. Of the currently clinically available methods, conventional echocardiography, electrocardiography, and tissue Doppler imaging lack the sensitivity required to detect the early stages of cardiomyopathy. The potential of newer echocardiographic methods, such as real-time 3- dimensional echocardiography, tissue Doppler imaging with myocardial strain, and strain rate imaging is, however, encouraging [67-70]. Also, minimally invasive methods, such as measurement of blood and serum markers of endothelial damage and biochemical cardiac markers, are promising [67].

^{123}I-Labeled Metaiodobenzylguanidine (Mibg) Scintigraphy

LVEF at rest is used by most oncologists to guide anticancer treatments. However, because of the compensatory reserve of the myocardium, which enables adequate ventricular output even in the presence of dysfunctional myocytes, LVEF at rest can underestimate actual cardiac damage. Some patients with normal resting LVEF show an abnormal response to exercise. Furthermore, study shows that the LVEF at rest correlates poorly with early myocardial damage as determined with endomyocardial biopsy [71–73]. A certain critical mass of cell damage must occur before LVEF begins to decrease. In fact, clinically overt cardiomyopathy is a late manifestation of progressive subclinical myocardial damage. Detection of myocardial injury before irreversible severe left ventricular dysfunction has occurred would be a logical approach. 123I-labeled mIBG scintigraphy is a technique that may provide such an approach [74, 75]. mIBG is a guanethidine analog that shares the same uptake, storage, and release pathway as norepinephrine. Unlike norepinephrine, mIBG is not metabolized by catechol-O-methyl transferase and monoamine oxidase and thus has longer residence in adrenergic receptors. When labeled with 123I, mIBG can be used to generate a scintigraphic image of the efferent sympathetic nervous innervations of the heart. The sympathetic nervous system uses norepinephrine as its

neurotransmitter and acts via adrenoceptors in the target tissue. A compensatory sympathetic drive, which increases the contractility, conduction, and heart rate, is activated in a condition of decreased myocardial performance. Neurohumoral responses to congestive heart failure primarily augment renin– angiotensin and sympathetic adrenergics to preserve organ perfusion. Vasoconstriction increases afterload and further lowers cardiac output. Therefore, patients with congestive heart failure have higher levels of circulating noradrenaline and plasma renin. These may contribute to vasoconstriction and progressive impairment of left ventricular function [76]. 123I-mIBG scintigraphy has been shown to have a good reproducibility and appears to be sufficiently sensitive to detect abnormalities of myocardial adrenergic innervations before left ventricular function is reduced. After intravenous administration of 123I-MIBG, the left ventricle myocardium can be visualized within a few minutes. This initial concentration, measured 15 min after injection, apparently depends primarily on blood flow. It reflects both the extravesicular and the intravesicular accumulation of 123ImIBG in cardiac neurons. The extravesicular concentration of 123I-mIBG decreases rapidly, whereas the intravesicular concentration remains relatively constant. The concentration plateau after 4 h, indicates the adrenergic neuron terminal concentration. Therefore, the 123I-mIBG uptake at 4 h after injection is used to explore specific neuron injury and impairment of the norepinephrine uptake function. 123I-mIBG uptake is often expressed semiquantitatively by drawing regions of interest over the myocardium and mediastinum for calculation of a heart-to-mediastinum count ratio. These relatively simple measures have been widely used in many studies, which support the usefulness of 123I-mIBG scintigraphy in predicting the severity and prognosis of heart failure. An association has been found between the heart-to-mediastinum ratio on the delayed image, the washout rate, brain natriuretic peptide serum norepinephrine concentration, and heart-type fatty acid binding protein, which is a marker for ongoing myocardial damage [77, 78]. Another report indicated that the heart-to-mediastinum ratio reflected myocardial contractile reserve [79].

CONCLUSION

From the above study it is concluded that biomarkers along with the newly developed techniques like planar multigated radionuclide angiography (MUGA), quantitative gated blood-pool SPECT (GBPS), 2- and 3-dimensional echocardiography, radiographic contrast angiography or cardiac MRI, conventional echocardiography, electrocardiography, and tissue Doppler imaging prevent damage to myocardium and can be used to treat various disorders which are associated with either increased in the level of biomarkers or some genetic fators.

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