



Clinical Assessment of Cardiovascular Disorders by Cardiac Biomarkers

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ABSTRACT

Cardiovascular diseases (CVD) are the primary cause of morbidity and mortality in the country. Therefore, Blood cardiac biomarkers (CB) have become progressively more accurate for estimating cardiac abnormalities for the last 40 years. Biomarkers are a significant tool to better identify high-risk individuals, to diagnose disease conditions promptly and accurately, and to efficiently prognosticate and treat patients with disease. Various biomarkers have been developed to assess cardiotoxicity which arises due to chemotherapy treatment, complications from anorexia nervosa, adverse effects of heavy metals intake, or an incorrectly administered drug such as bupivacaine. Biomarkers like miRNAs are potentially new biomarker agents having the benefits of both tissue and disease specific expression mark. Secreted miRNAs are protected from degradation by ribonucleases, RNA-binding proteins and lipid vehicles. All of these qualities equate to miRNAs being very attractive as diagnostic biomarkers. Another developed biomarker is Glycogen phosphorylase isoenzyme BB, Soluble CD₄₀ ligand, ST₂, ET₁/CTproET₁, exosomes as biomarkers of disease. Furthermore, new molecular diagnostics techniques are also available to access various cardiac related maladies like cardiomyopathy, heart failure and arrhythmia. Like Endomyocardial biopsy (EMB), Immunoassay, Histological Analysis, Immunohistochemical Examination on Intramyocardial Inflammation, Molecular Virology for detection of Myocardial Infarctions, Indirect Immunofluorescence testing of Autoimmunity, Proof of disease-related gene mutations for Cardiomyopathies, Diagnostic-based treatment decisions are the various techniques to access cardiac disorders.

Keywords: Cardiac markers, miRNA, Glycogen phosphorylase isoenzyme BB, Endomyocardial biopsy, Immunoassay, Immunohistochemical Examination, Indirect Immunofluorescence.

INTRODUCTION

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 hemodynamic 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 can be due to over expression of the free radicals which results in oxidative stress, along 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 consists of a wide range of cardiac effects from small changes in blood pressure and arrhythmias to cardiomyopathy.

Biomarkers are a significant tool to better identify high-risk individuals, to diagnose disease conditions promptly and accurately, and to efficiently prognosticate and treat patients with disease. The term Biomarkers (biological markers) means "any measurable and quantifiable biological parameters which contain specific enzyme concentration, hormone concentration, specific gene phenotype distribution in a population, presence of biological substances which provide 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" (figure 1). In 2001, NIH working group synchronized 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".⁴

An ideal biomarker would have several properties including high sensitivity and specificity, quick release into blood or serum for easy detection, a low detection limit, a reasonable assay time and the ability to be measured quantitatively in a cost-effective manner.^{5,6}

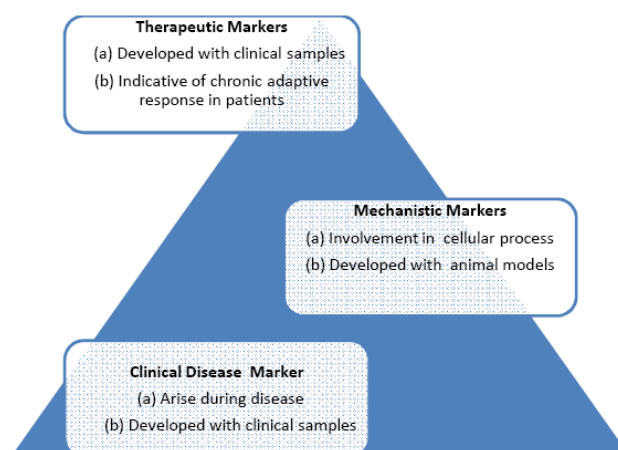


Figure 1: Represent Role of Biomarkers in Assessing disorders.⁷

ROLE OF BIOMARKERS IN ASSESSMENT OF CARDIOTOXICITY

Cardiovascular diseases (CVD) are the primary cause of morbidity and mortality in the United States.⁸ Blood cardiac biomarkers (CB) have become progressively more accurate for estimating cardiac abnormalities during the past 40 years. In the beginning, with the focus on myocardial infarction (MI), the use of creatinine kinase-MB (CKMB), was first described in 1972, was a key step in the advancement of a highly cardiac-specific biomarker. The introduction of cardiac troponin (cTn) assays in 1989 was the next foremost advancement, and subsequent refinement of the assays now has the definition of acute myocardial infarction (AMI) centered on it. This development ironically has brought significant difficulties to the critical care physician who deals with multi-organ failure rather than the patient presenting to the emergency department with chest pain or single-organ pathology. The recent incursion of high-sensitivity cardiac troponin (cTn) has replaced the fourth-generation cTn assays. Whilst cardiac ischemia/ infarction is the most widespread cause of cardiac injury with biomarker development reflecting this, the search for more significant biomarkers now includes Cardiac Biomarkers for inflammatory processes and myocardial wall stress occurs as a result of pressure or volume overload where assessment expands beyond myocardial necrosis (figure 2). The important role of C-reactive protein (CRP) as a prognostic marker is an example of the former while natriuretic peptides are now accepted as clinically useful markers of cardiac stress. In the critical care setting, the challenge of confounding factors brings about interpretation difficulties. Even so, an understanding of the commonly used CB can be very helpful for cardiac evaluation of the critically ill patient.⁹

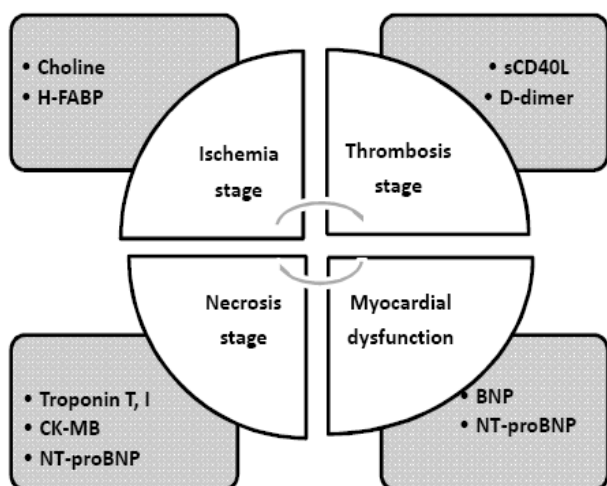


Figure 2: Biomarkers associated with various pathophysiological processes on developing acute myocardial infarction (AMI). BNP, Brain natriuretic peptide; CK-MB, Creatine Kinase-MB; NT-proBNP, N-terminal proBNP; H-FABP, Heart Type- fatty acid binding protein.⁶

RECENT ADVANCES IN CARDIAC BIOMARKERS

A number of added new clinical markers have also been studied recently. Several of these demonstrate promising results as early prognostic and diagnostic markers.

miRNAs biomarkers for early assessment of subclinical myocardial injury

miRNAs are potentially promising new biomarker agents having the benefits of both tissue and disease specific expression mark.¹⁰ The miRNAs sequences are stable in body fluids, such as blood plasma and serum, urine, saliva, amniotic fluid and pleural fluid. Secreted miRNAs are protected from degradation by ribonucleases, RNA-binding proteins and lipid vehicles.^{11, 12} All of these qualities equate to miRNAs being very attractive as diagnostic biomarkers. The involvement of miRNAs in the pathological process of the cardiovascular system is a rapidly emerging area of research. miRNAs expression varies in different heart disease states, suggesting the involvement of etiologic factors impacting on miRNAs expression.¹³ Interestingly, the promoters of RNA polymerase II often contain toxicologically significant enhancer regions¹⁴, which show that miRNAs play an important role in responding to cellular stresses and gene changes when exposed to toxic substances. miRNAs are stable in body fluids and can readily be accessed to monitor and diagnose patho-physiological organ-specific diseases. *miR-208a* and *miR-499* are expressed particularly in heart muscle tissue,^{15,16} whereas *miR-1* and *miR-133* are also found in injured skeletal muscle.^{17, 18} These miRNAs are highly expressed in the cardiac muscles and are involved in heart development and myocyte differentiation. The cardiac muscle cells express *miR-126-3p*, *miR-30c* and *miR-26a* in addition to *miR-1*, *let-7* and *miR-133*.¹⁹ Expression of specific miRNAs is vital for fully functional cardiac development. Abnormal *miR-1* levels are associated with cardiac defects, like arrhythmias, ventricular septation dysfunction, cardiac hypertrophy and myocyte hyperplasia. Arrhythmias are also dependent on altered *miR-133* expression, whereas *miR-21*, *miR-133*, *miR-195* and *miR-208* are involved in cardiac hypertrophy. The development of a translational non-clinical to clinical miRNAs-based biomarker to assess for the cardio toxic effect of a substance should be done by this newly developed biomarker.

Caveolae

Caveolae are flask-like invaginations²⁰ that create signalling micro-domains of the plasma membrane enriched with cholesterol, sphingolipids, the marker protein caveolin, and the coat protein cavin.^{21, 22} Caveolins have three iso-forms (caveolin 1-3) and cavin consists of four iso-forms (cavin 1-4). Caveolin-3²³ and cavin-4²⁴ are expressed predominantly in cardiac muscle and have been identified as important proteins involved in cardiomyopathy.^{25, 26} In cardio-myocytes, 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.

Glycogen phosphorylase isoenzyme BB

Glycogen phosphorylase is a dimeric enzyme (~97 kDa) of glycogenolysis which is composed of two identical subunits like BB and MM iso-enzymes which are found in the human heart; however, the BB isoenzyme is the most important myocardial enzyme. It is known that glycogen phosphorylase isoenzyme BB (GPBB, 177 kDa) is highly sensitive for the diagnosis of AMI within 4 h after the onset of chest pain. In several clinical studies, GPBB rapidly increased within 2–4 h after the onset of chest pain, indicating that it is a useful marker for early risk evaluation.²⁷⁻²⁹ This is an ischemic marker with an early release from the injured myocardium, which has only been investigated in acute coronary syndromes and in patients with miscellaneous diseases or those undergoing different kinds of surgery.³⁰⁻³² It is also a sensitive marker for detection of perioperative myocardial injury in patients undergoing coronary artery bypass grafting. In fact, it is considered as the most promising marker among the recently proposed new markers for early diagnosis of AMI.³³

sCD₄₀L

Soluble CD₄₀ ligand (sCD₄₀L) has been proposed as an added biomarker that indicates platelet activation in acute myocardial infarction (AMI). It is recognized that the CD₄₀ ligand is a transmembrane protein which is expressed on platelets. After platelet activation, it is rapidly released in a soluble circulating form. Both membrane-bound CD₄₀L and sCD₄₀L forms interact with the CD₄₀ receptors, which are present on B cells, monocytes and macrophages.^{34, 35} It has been established that sCD₄₀L concentration is significantly higher in AMI patients than in normal populations, which shows that increased sCD₄₀L levels is associated with high risk of cardiac symptoms.³⁷ According to other reports, sCD₄₀L levels ranged from 0.03 to 4 µg / l in control groups; however, the levels of sCD₄₀L were 3–6 µg/l in patients with acute chest pain.³⁶ Therefore, sCD₄₀L concentration may identify AMI patients and be useful as another indicator of ACS patients.

Choline

Choline is the major metabolic product produced by phosphodiesteric cleavage of membrane phospholipids. A number of study suggests that early ischemic membrane damage and phospholipids breakdown by phospholipase could induce the release of choline into plasma by choline regulatory systems.^{37, 38} An increased in the level of choline has been considered as an important indicator of cardiac death, cardiac arrest and heart failure.³⁷ Whole blood choline and plasma choline are quite independent of various factors like age, gender, etc; however both are predictive for cardiac death and heart failure. The

standard measurement of choline in whole blood will be necessary for identifying high-risk in AMI patients.

ST₂

The study suggests that ST₂ is a good biomarker for heart failure induced by mechanical stress.^{39, 40} ST₂ is also known as interleukin-1 receptor-like protein which is expressed by mechanical stress and released into serum. ST₂ only appears as a soluble fraction and is increased in the mouse model. In mouse studies, serum levels of ST₂ were correlated with NT proBNP, and both markers would be useful to predict heart death or failure.⁴⁰ In human studies,³⁹ the levels of ST₂ were significantly higher in AMI patients. The study suggested that soluble ST₂ might be a useful biomarker.

ET₁/CTproET₁

Endothelin-1 (ET₁) and C-terminal of pro-endothelin-1 (CTproET₁) have been accounted to be prognostic of heart death or failure after AMI. Several studies suggest that levels of ET₁ and CTproET₁ were increased in heart failure.^{41, 42} ET₁ is a vasoconstrictor peptide which was cloned and isolated from vascular endothelial cells. It is interesting to note that plasma level of unstable ET₁ and CTproET₁ which is stable were associated with NT proBNP in AMI patients, which indicate the predictive value of post-AMI. A study suggested that multimarker strategy with CTproET₁ and NT proBNP would provide greater accuracy.

Exosomes as biomarkers of disease

Exosomes are secretory membranous vesicles of 40–100nm diameter which are secreted in the endosomal compartment and released through fusion of multivesicular bodies with the plasma membrane in comparison to shedding vesicles which is ranging from 100–1000nm.⁴³ Recent studies show that exosomes are released from multiple cell types which contain protein and RNA species, and have been exploited as a novel reservoir for disease biomarker discovery. The molecular content including proteins, in the form of exosomes are heavily dependent on the tissue/cell-type from which it is derived. Gupta et al showed shed micro vesicles and exosomes were released from cardiomyocyte, which were not considered to be secretory cells earlier HSP₆₀ were tripled upon mild hypoxia.⁴⁴ Waldenstrom et al focused on the mRNA content of exosomes released from cardiomyocyte under normal conditions and identify 1520 mRNA by microarray analysis.⁴⁵ MicroRNA (miRNAs) has been found in current research to be a close regulator of messenger RNA (mRNA) translation and found to be confined from degradation by their encapsulation into exosomes. miRNAs are short non-coding oligonucleotides of approximately 20–26 nucleotides in length with approximately 650 identified in the human genome. Their mechanism of regulating mRNA translation involves the miRNAs interacting with the 3' untranslated region of the target mRNA, leading to target degradation or gene silencing.⁴⁶ There has been evidence supporting the use



of miRNAs as viable circulating biomarker for myocardial injury.⁴⁷

CLINICALLY RELEVANT CARDIAC MARKERS

Fatty acid binding protein (FABP)

Fatty acid binding proteins (FABPs) small proteins located in the cytoplasm which facilitates transport of fatty acids and other lipids within the cell.⁹ Heart-type FABP (H-FABP) is a sensitive marker of myocyte damage and unlike troponin which is released by both ischemia and necrosis. It is released more rapidly than troponin, although the introduction of hs-cTn will overcome the advantage of FABP in this regard. H-FABP is released from the damaged cell within 1-3 hours, returning to normal by 12-24 hours. It is now used as a diagnostic test of AMI in many countries and also has good data to support its use in prognostication from MI.^{48, 49} The diagnostic sensitivity of H-FABP for cardiac injury is 93.1%, higher than CK-MB and cTn.⁵⁰

C-reactive protein (CRP)

C-reactive protein (CRP) is a non-specific acute-phase reactant protein produced in the liver.⁵¹ It is interrelated with diverse functions related to immune reactivity including complement activation, innate immunity and phagocyte stimulation. It has been found to be a reliable marker for various CVD-related pathologies like atheromatic plaque vulnerability, atherosclerosis and coronary artery disease, coronary vasospasm, left ventricular dysfunction, angina pectoris and myocardial infarction. CRP levels have been found to be related to levels of cardiac enzymes and troponin I, while in some cases it was found to be a better marker of CVD than troponin T.⁵² CRP plays an important role in myocardial and cerebral infarct growth and has been therefore targeted by inhibitors to induce a cardio-protective effect. Its consistency has several limitations as human CRP levels significantly vary, depending on ethnicity, gender, genetics and is also associated with obesity and weight loss. In addition, it has been expressed as an indicator/ marker for non-cardiac related pathologies such as anastomotic leakage, systemic lupus erythematosus (SLE), and dementia.^{53, 54, 55}

Myoglobin

Myoglobin is a heme protein found in skeletal and cardiac muscle that has attracted considerable interest as an early marker of MI. Its low molecular weight (17.8 kDa) accounts for its early release profile: myoglobin typically rises 2-4 hours after onset of infarction, peaks at 6-12 hours, and returns to normal within 24-36 hours.⁵⁶ Physiologically, myoglobin is found in all muscle tissues, indicating that it lacks specificity for cardiac injury or damage.^{35, 57} Although myoglobin in serum is rapidly secreted as early as 1-4 h after the onset of AMI, the specificity of myoglobin is low because of its abundant presence in myocardial and skeletal muscle injury and other diseases. The level of myoglobin increase in AMI,

Vigorous exercise, Rhabdomyolysis, Shock, Renal failure, Muscular dystrophy. Rapid myoglobin assays are available, but overall, they have a lack of cardio specificity. Serial sampling every 1-2 hours can increase the sensitivity and specificity; a rise of 25-40% over 1-2 hours is strongly suggestive of acute MI. However, in most studies, myoglobin only achieved 90% sensitivity for acute MI, so the negative predictive value of myoglobin is not high enough to exclude the diagnosis of acute MI.⁵⁶

Matrix Metalloproteinases (MMP)

MMP are endogenous zinc dependent endopeptidases required for structural integrity of extracellular matrix of myocardium and are also a member of proteases that together with other proteases, such as cathepsins and elastase, play a key regulatory role in breakdown of connective tissues and are important in bone remodelling in both physiology and in a number of pathologies which include cancer, fibrosis and CVD, the menstrual cycle, repair of tissue damage.⁵⁸ TIMP (Tissue Inhibitors of Metalloproteinases) regulates MMP. MMPs may degrade myocardial ECM leading to the development of LV dilatation and heart failure and their inhibition in experimental models of AMI has been associated with reduced LV dilatation and wall stress.⁵⁶ MMP activities have been implicated in a large number of diverse cardiac and vascular pathologies which include cardiomyopathy, atherosclerosis, aneurism, myocarditis, hypertension and viral heart disease.⁵⁸ In a study of patients with acute myocardial infarction, TIMP-1 and MMP-9 correlated with echocardiographic parameters of LV dysfunction and remodelling after AMI and identified patients at risk of subsequent LV remodelling and associated with severe extensive CAD.⁵⁶

Placental Growth Factors (PGF)

Placental Growth Factor is a member of VEGF (vascular endothelial growth factor) subfamily, which plays a major role in angiogenesis (blood vessel formation) and vasculogenesis (in the development of vascular system), in particular during the embryogenesis. Placental growth factor is expressed within human atherosclerotic lesions which is associated with plaque inflammation and neovascular growth. Recent studies proved that the function of different inflammatory markers such as hs CRP, sr amyloid A, IL-6 show increased in the level of markers during acute coronary syndrome (ACS) but also show some adverse effects. Recent report suggested that, PGF is up regulated in all forms of atherosclerotic lesions. PGIF induces vascular smooth muscle cell growth, recruits macrophages into atherosclerotic lesions, and up regulates production of TNF- α , monocyte chemo tactic protein 1 by macrophages, pathological angiogenesis. Plasma PIGF levels may be an independent inflammatory biomarker of poor result in patients with suspected ACS.⁵⁶

Pregnancy associated plasma protein alpha (PAPP- α)

PAPP- α was formerly identified in the serum of pregnant women and is produced by placental tissue. The level of



Circulating PAPP- α increase during pregnancy and is used in the foetal diagnosis of Down syndrome. Recently PAPP- α has been identified in non-placental tissues. PAPP- α is a high-molecular-weight, zinc-binding metalloproteinase, which act as a specific protease of IGF binding protein-4 (IGFBP- 4). The study show that, using specific monoclonal antibodies, PAPP- α is abundantly expressed in both eroded and ruptured coronary plaques, but not in stable plaques, in patients who have died suddenly of cardiac causes. Furthermore, evidence suggests that PAPP- α play an essential role in the development of atherosclerosis and successively plaque instability in ACS patients.⁵⁶

RECENT ADVANCES IN MOLECULAR DIAGNOSTICS

Over the years, several imaging modalities have been developed that vary significantly in precision, ease of use, availability and costs. Left ventricular ejection fraction (LVEF) can 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. Currently, clinically available methods include conventional echocardiography, electrocardiography and tissue Doppler imaging lack the sensitivity required to detect the early stages of cardiomyopathy. Therefore, recent advances have been made in molecular diagnosis of various heart related disorders like Endomyocardial Biopsy (EMB), Histological analysis, Immunohistochemical Examination on Intramyocardial Inflammation, Molecular Virology for Detection of Myocardial Infections, Indirect Immunofluorescence testing of Autoimmunity, Immunoassays, Profiling Technologies for miRNAs or Gene Expression—The Way to Organ-Specific and Systemic Diagnosis, Proof of Disease-Related Gene Mutations for Cardiomyopathy, Diagnostic-Based Treatment Decisions are the so-called techniques which is used currently.

Endomyocardial Biopsy (EMB)

Since patho-physiological changes of acquired infectious and non-infectious heart muscle diseases, that can be treated particularly by anti-viral or anti-inflammatory medication, which occur at cellular and sub-cellular levels, various imaging techniques such as Cardiac Magnetic Resonance (CMR) or echocardiography provide only non-invasive tissue characterization but fail in revealing the true underlying causes of a disease.^{59, 60, 61} Invasive elimination of a sufficient number of tissue samples by EMB is always essential when a correct analysis cannot be achieved by other clinical methods and is influencing the following treatment. EMB is compulsory in so-called myocarditis and Dilated cardiomyopathy (DCM) of unknown aetiology since the diagnosis of myocarditis and other inflammatory diseases (inflammatory cardiomyopathy, cardiac sarcoidosis, giant cell myocarditis), viruses or storage diseases (amyloidosis) is not possible without their direct proof in myocardial

tissue.⁶² The use of LV EMB to study cardiomyopathy is presently discouraged because it is considered riskier. Similarly, a recent study shows that around 4221 patients undergoes diagnostic EMB during a period of 28-year confirmed that biventricular EMB has a low major complication rate (perforation with or without cardiac tamponade, embolization) of 0.33% for LV EMB and 0.45% for RV EMB and no patient were died. According to these studies, LV or RV EMB is a secure method with very low transient difficulties. The histological examination of paraffin sections by different staining protocols (HE, PAS, Azan) identify myocardial cell death, scars, fibrosis, disarrays, cardiomyocyte changes, pathological vascular conditions, granulomas and inflammatory cell differentiation. Storage disorders such as amyloidosis, iron deposits, glycogen and others can be expelled or specified by other staining, e.g., Immunohistochemical differentiation of amyloid subtypes and optional electron microscopically analyses. The EMB diagnosis of myocarditis was based on histomorphological criterion according to the Dallas classification.^{63, 64} Routine Immunohistochemical diagnostics are based on the use of antibody-supported detection of inflammatory infiltrates (lymphocytes, macrophages, memory cells, B-cells) and enhanced the expression of tissue activation marker such as adhesion molecules (HLA, ICAM, VCAM) or fibrotic proteins like collagens on cryofixed tissue section, following quantitative digital imaging analysis. The use of these sensitive techniques detecting the most common cardio tropic viruses (such as enteroviruses, adenoviruses, erythrovirus, human herpes virus 6, Epstein-Barr virus and in the Far East also hepatitis C) reveals a virus infection in up to 73% of patients who are biopsied under the clinical suspicion of myocarditis or DCM. The clinical significance is clearly demonstrated only for some cardio tropic viruses.

Immunohistochemical Examination on Intramyocardial Inflammation

Immunohistochemical diagnostics are based on application of specific primary antibodies on cryofixed tissue section and followed by the detection of coupled primary antibody with the help of secondary antibody.⁶⁵ The secondary antibody is conjugated with an enzyme to form complex which produces a precipitating coloured complex with the help of staining solution. Coloured immune-spots obtained are counted digitally by application of traditionally digital imaging analysis software for calculating various parameters like area fractions, numbers of immunospots and area of myocardial tissue.⁶⁶ The digital imaging system consists of a microscopic unit, a digital camera and supporting analyzing software. Immunohistochemical analysis of intra-myocardial inflammation and activation of different tissue fractions (myocytes, capillary) in further biopsies supports the results achieved by histology. Immunohistochemical analyses are carried out on frozen sections (two EMB) in order to allow detection of elevated inflammatory cell subsets together with non-



paraffin staining antibodies, e.g. CD3, CD11a (LFA-1), CD11b (MAC-1), CD45RO (memory or activated lymphocytes), Perforin positive cytotoxic lymphocytes and increased in the expression of adhesion molecules such as CD54 (ICAM), CD106 (VCAM) and HLA-1 were used as marker for tissue activation. Perforin positive, cytotoxic cells were identified in biopsies in acute and chronic phases of infection. Increased in the level of Perforin positive cells in myocardial tissue are related with more number of lesions of cardiomyocyte and poor clinical prediction of affected patients.⁶⁷ Introduction of HLA-1 and CD54 in routine biopsy diagnostics progresses the prediction of inflammatory processes in myocardium without on-site identification of infiltrative cellular foci.⁶⁶ Patients with detectable intra-myocardial inflammation are classified as borderline myocarditis due to Immunohistochemistry. Newly, investigative markers for cardiomyopathy are the micro vessel density (MVD) and the quantitative assessment of fibrosis. MVD in endomyocardial biopsies is found to be a critical marker in the development of heart muscle diseases. Diagnostics of MVD is carried out by Immunohistochemistry of endothelial surface marker CD31. Due to chronic inflammation or viral infections of myocardium, the level of MVD reduces which results in malfunctioning of myocardium and induces atrophy or hypertrophy of cardio-myocytes. Myocardial fibrosis detected by CMR is an independent and incremental predictor of mortality and sudden cardiac death in DCM patients.⁶⁸ Correlation of fibrotic areas in myocardial tissue for the risk stratification of acquired cardiomyopathy requires additional study.

Immunoassays

The immune systems defend the body from infection by generating and maintaining barriers that prevent bacteria and viruses from entering in the body. If pathogens infiltrate these barriers, and gets into the body, the innate immune system is equipped with specialized cells that detect, and eliminate, the invader before it is able to reproduce, potentially causing severe injury to the host. The innate immune system protects the host by establishing humoral, chemical and cellular barriers to infection. Inflammatory response is produced by chemical factors such as specialized chemical mediators, called cytokines. The level of Cytokine in peripheral blood corresponds to systemic situation in patients initiated by various factors like infections or inflammatory processes. Cytokines or chemokines are estimated in blood serum for categorization of present immune response. High level of interferon- β in serum results in elimination of enteroviruses from myocardial tissue and is associated with reduced mortality rate in cardiac patients.⁶⁹ Immunoassays on cardiac auto-antibodies or cellular hormones like adiponectin⁷⁰ are excellent tools for screening clinical cause of cardiomyopathy.

CONCLUSION

From the above study it is concluded that newly developed biomarkers such as miRNAs, choline, exosomes as biomarkers of disease, glycogen phosphorylase isoenzyme BB, caveolae, sCD40L, ST2, ET1/CTproET1, along with the newly diagnostics techniques like Endomyocardial biopsy (EMB), Histological analysis, Immunohistochemical Examination on Intramyocardial Inflammation, Molecular Virology for Detection of Myocardial Infections, Indirect Immunofluorescence testing of Autoimmunity, Immunoassays, Proof of Disease-Related Gene Mutations for Cardiomyopathy, Diagnostic-Based Treatment Decisions can be used to treat various disorders which are associated with either increased in the level of biomarkers or may be due to some pathological alterations.

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