Novel Invasive and Noninvasive Cardiac-Specific Biomarkers in Obesity and Cardiovascular Diseases

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Abstract

Cardiovascular disease (CVD) is the leading cause of fatality and disability worldwide regardless of gender. Obesity has reached epidemic proportions in population across different regions. According to epidemiological studies, CVD risk markers in childhood obesity are one of the significant risk factors for adulthood CVD, but have received disproportionally little attention. This review has examined the evidence for the presence of traditional cardiac biomarkers (nonspecific; lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, creatine kinase, myoglobin, glycogen phosphorylase isoenzyme BB, myosin light chains, ST2, and ischemia-modified albumin) and novel emerging cardiac-specific biomarkers (cardiac troponins, natriuretic peptides, heart-type fatty acid-binding protein, and miRNAs). Besides, noninvasive anatomical and electrophysiological markers (carotid intima-media thickness, coronary artery calcification, and heart rate variability) in CVDs and obesity are also discussed. Modifiable and nonmodifiable risk factors associated with metabolic syndrome in the progression of CVD, such as obesity, diabetes, hypertension, dyslipidemia, oxidative stress, inflammation, and adipocytokines are also outlined. These underlying prognostic risk factors predict the onset of future microvascular and macrovascular complications. The understanding of invasive and noninvasive cardiac-specific biomarkers and the risk factors may yield valuable insights into the pathophysiology and prevention of CVD in a high-risk obese population at an early stage.

Keywords: cardiovascular diseases, cardiac-specific biomarkers, cardiac troponins, heart rate variability, natriuretic peptides, childhood obesity

Introduction

OBESITY IS BECOMING a global epidemic in both children and adults. It is associated with an increased risk of morbidity and mortality as well as reduced life expectancy; with numerous comorbidities, such as cardiovascular diseases (CVDs), type 2 diabetes, and hypertension. Obesity is an independent risk factor for CVD, and CVD risks have also been documented in obese children. Excess reactive oxygen species (ROS) production plays a pivotal role in oxidative damage¹ and may contribute to the onset and progression of these pathologies of CVDs^{1,2} (Fig. 1). Although an initiating factor is still elusive, development of CVD within all these tissues is a complicated cycle of lipid and glucose toxicity, which leads to the development of oxidative stress, chronic inflammation, and insulin resistance.

There are eight critical risk factors for heart disease and stroke that play a significant role in the progression of CVD according to The National Heart, Lung, and Blood Institute of the U.S. National Institutes of Health (Fig. 2).³ Along with nonmodifiable risk factors, such as age and family history, six of the factors are modifiable: the biological factors, including hypertension, diabetes, and abnormal lipid profile; and the lifestyle factors, including tobacco use, physical inactivity, and obesity. CVDs involve the cardiovascular system; the heart, blood vessels, and the circulatory system (Fig. 3).⁴ World Health Organization reports that the prevalence of CVD will double by 2020, passing HIV/AIDS infection and causing more than 20 million CVD-related deaths globally.⁴ CVD is the leading cause of fatality globally regardless of gender⁵ and accounts for 48% of noncommunicable diseases,^{3,6} although estimates predicted that 90% of CVD is avertable.⁷

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FIG. 1. The major modifiable risk factors—obesity and metabolic syndrome—redox imbalance essential predictors of CVD. CVD, cardiovascular disease. Color images are available online.

The pathological processes and risk factors of atherosclerosis have been shown to begin during early childhood.⁸ Among people with diabetes, CVD is the most life-threatening complication; diabetic population showed the leading cause of cardiovascular-related death (2–4-fold higher incidence) than nondiabetics.^{9–11}

Visible abdominal obesity is a crucial sign of CVD along with the elevated levels of very low-density lipoprotein (LDL), triglycerides, and low levels of high-density lipoprotein cholesterol (HDL-C).¹² Similarly, quantification of epicardial adipose tissue and assessment of coronary computed tomography (CT) angiography can establish a correlation between coronary artery disease (CAD)¹³ and function as clinically essential biomarkers in acute coronary syndrome (ACS).¹⁴

Biomarkers are promising of tools for use in the overall management of the screening, diagnosis, and treatment of

FIG. 3. Classification of CVDs involving in the blood vessels (vascular disease) and heart (heart disease). Color images are available online.

at-risk subjects/patients.^{15,16} This review has examined the evidence for the presence of traditional cardiac biomarkers [nonspecific; lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), myoglobin, glycogen phosphorylase isoenzyme BB (GPBB), myosin light chains, ST2, and ischemia-modified albumin (IMA)] and novel emerging cardiac-specific biomarkers [cardiac troponins (cTn), natriuretic peptides (NPs), heart-type fatty acid-binding protein (H-FABP), and miRNAs]. Besides, noninvasive anatomical and electrophysiological markers [carotid intima-media thickness (CMT), coronary artery calcification (CAC), and heart rate variability (HRV)] in CVDs and obesity are also discussed. A timeline of the history of the development of cardiac biomarkers of myocardial injury, necrosis or ischemia, and hemodynamic stress and its sensitive assays/biomarkers are summarized in Fig. 4.^{17–38}

The understanding of invasive and noninvasive cardiac-specific biomarkers and the risk factors may yield valuable insights into the pathophysiology and prevention of CVD in a high-risk obese population at an early stage.

Risk Factors for CVD

There are numerous risk factors associated with coronary heart disease (CHD) and stroke. A few risk factors, such as family history, cannot be modified, while other risk factors, such as high blood pressure, can be modified with treatment. Obesity, diabetes, sedentary lifestyle, and diet are modifiable risk factors (Fig. 2).

Cardiovascular disorders risk in the young adolescent population

CVD considered as a disease of adulthood, as mentioned above, the risk factors for CVD are present during childhood and persist into adulthood. Obese children have a higher chance to become obese adults.³⁹ Severe health conditions in adult, including type 2 diabetes and heart disease are associated with and have a higher risk with obesity.^{40,41} Obese

FIG. 2. Classification of modifiable and nonmodifiable cardiovascular risk factors. Color images are available online.

FIG. 4. The developmental timeline history of cardiac biomarkers of myocardial injury, necrosis or ischemia, and hemodynamic stress and its sensitive assays. Color images are available online.

children are more likely to experience severe obesity and disease risk factors in adulthood.⁴²⁻⁴⁴ Dietary modifications and therapeutic lifestyle changes may reduce obesity and its associated complications,⁴⁵ which is essential because the upsurge in deskbound behavior in children and youth is an emerging risk factor for cardiometabolic disorders.⁴⁶

Epidemiological studies show that atherosclerosis, the forerunner of CVD, initiates in childhood. Overweight adolescents ran a relatively higher risk end of mortality from CHD, after 55 years of follow-up, independent of adult weight.⁴⁷ Obese youths (7-9 years), majority having aortic intimal lesions and more than 50% of the right coronary arteries⁴⁸ is shown in the Pathobiological Determinants of Atherosclerosis in Youth Study. Cardiovascular risks among adolescents are poorly understood. The increase in unattended risk factors and the obesity pandemic have created calculable cardiovascular disorder risk in the young adolescent population.

Gender differences in obesity, diabetes, and CVDs

CVD has traditionally been viewed as a male disease; however, the relative risks for diabetes, obesity, and hypertension morbidity and mortality, major risk factors for CVD,

are higher for women. In the general population, obesity has adverse effects on most of the major CVD risk factors; however, despite these adverse effects on CVD risk factors and increasing the prevalence of CVD, the physiological disparities between the sexes likely contribute to differences in disease onset, susceptibility, and prevalence and treatment responses.⁴⁹ In the United States, obesity affects more women (~36%) than men (32%); as obesity is associated with a loss of cardioprotection, CVD occurs at an earlier age in obese women than in lean women.⁵⁰ The cardioprotection generally observed in women <55 years of age is lost in the milieu of obesity and metabolic syndrome.^{51,52} Interestingly, obese women also develop more obesity-related conditions, such as hyperlipidemia, insulin resistance, and type 2 diabetes mellitus, than obese men, and these conditions are associated with more severe outcomes in women.⁵¹

Metabolic syndrome may cause sympathetic overactivity and hypertension

Obesity, a significant risk factor for diabetes and CVD, is associated with dysregulation of autonomic function and

overactivity of the sympathetic nervous system (SNS) (Fig. 1), which may contribute to glucose intolerance and insulin resistance.^{53–58} Insulin acts on neurons in the arcuate nucleus of the hypothalamus and activates the sympathetic nerve.⁵⁹ The sympathetic activity is also controlled by insulin action, which decreases during fasting and increases in response to raised insulin levels following a mixed meal/during a euglycemic hyperinsulinemic clamp. Sympathetic overactivity in obesity can also contribute to cardiac arrhythmia, left ventricular (LV) diastolic dysfunction, LV hypertrophy, and heart failure (HF).⁶⁰ The degree of insulin resistance measured under obese conditions reflects autonomic dysfunction and is a predictor of CVD.

Dyslipidemia and hypertension-related cardiovascular risks

Dyslipidemia is a metabolic abnormality leading to a persistent increase in the plasmatic concentration of cholesterol and triglycerides (Fig. 1). All lipid-related abnormalities are responsible for cardiovascular mortality, fatal myocardial infarction (MI), and brain stroke.⁴ Pediatric groups prevalently suffer from diseases such as dyslipidemia/hyperlipidemia, atherosclerosis, familial hypercholesterolemia, hypertriglyceridemia, and diabetes. Physiological biomarkers are based on measuring the levels of critical components of lipid homeostasis, such as the triglyceride-to-HDL ratio, lipoprotein/cholesterol ratio, lipid/lipoprotein ratio, LDL cholesterol (LDL-C) level, HDL and apolipoprotein levels, lipoprotein and LTP ratio, sphingolipids, omega-3 Index, and ST2 level.⁶¹ Low levels of HDL-C independently contribute to CV risk and are associated with an increased risk of CHD, but HDL is protective against atherosclerosis.⁶²

Hypertension accounts for an estimated 54% of all strokes and 47% of all ischemic heart disease events worldwide.⁶³ Hypertension is one of the most critical risk factors for almost all varieties of premature CVD,^{64,65} including stroke, CAD, HF, atrial fibrillation (AF),⁶⁶ and peripheral vascular disease acquired over a lifetime; it is more common than dyslipidemia, diabetes, and cigarette smoking, which are the other major risk factors. The risk for both coronary disease and stroke increases progressively with incremental increases in blood pressure above 115/75 mmHg, as shown in numerous epidemiological studies.⁶³

Hypertension, often combined with obesity and dyslipidemia, is one of the foremost essential predictors of CVD^{67,68} (Fig. 1). Childhood obesity, hypertension, and dyslipidemia are modifiable antecedents of adult CVD.⁶⁹ Hypertension occurs in 3%–5% of children in the United States; among obese children, the percentage is much higher (up to 25%).⁶⁸ Hypertriglyceridemia and low high-density lipoprotein (HDL) cholesterol are the dyslipidemias most likely to occur in obese youths.^{69–73} Almost 17% of obese adolescents in the United States have abnormal non-HDL-C values; severe obesity correlates with high total cholesterol (10% with overweight to 19% with class III obesity), low HDL-C ($\leq 19\%$ with class III obesity), and high triglycerides (29% with class III obesity).^{69,70,73} In addition to the conventional clinic-based risk factors used to predict preclinical CVD, lipid measurements and the hypertension status in obese youth may affect the independent prediction of adult-onset CVD.

Metabolic syndrome—oxidative stress, inflammation, and adipocytokines

The metabolic syndrome is a common and complex disorder combining obesity, dyslipidemia, hypertension, and insulin resistance (Fig. 1). It is a primary risk factor for diabetes and CVD.^{74–76} Free radicals appear to be essential modulators of atherosclerotic disease in all stages of development and progression.⁷⁷ Physiologically, they are signal transducers in cell communication and homeostasis, including vascular gene expression and cell interactions.⁷⁸ Uncontrolled generation of free radicals leads later to disease. Oxidative stress may be a predictive marker for CVD.⁷⁹ Obese LDL receptor-deficient mice were associated with increased oxidative stress and impaired HDL-associated antioxidant defense associated with accelerated atherosclerosis due to increased macrophage infiltration and accumulation of oxidized LDL in the aorta.⁸⁰ Oxidative stress correlates with ventricular remodeling and progressive dilation leading to end-stage HF; further neuroendocrine system, ROS, and inflammatory cytokines are essential regulators of the remodeling process.^{79,81,82} The primary sources of CVD-ROS are mitochondrial NADPH oxidases (NOX), lipoxygenases, and myeloperoxidases (MPOs).⁸³ It has been shown that increased renin/angiotensin/aldosterone system activity stimulates NADH/NOX.^{84,85} The critical role of oxidative stress in cardiovascular pathophysiology has encouraged quantification of ROS as a promising biomarker reflecting the disease process. However, the transitory nature and short half-life confound in the measurements of ROS by standard approaches such as spin-trapping⁸⁶ method. Different markers [isoprostanes, malondialdehyde, MPO, OxLDL, and oxidative protein post-translational modifications (nitrotyrosine, S-glutathionylation, S-sulfhydration)] are elevated in the presence of multiple CV risk factors.^{86–88}

Epicardial and pericardial adipose tissues are local energy sources that facilitate the contractile activity of the heart by releasing fatty acids (lipolysis) under standard conditions.^{89–91} However, increased epicardial fat (EF) and pericardial fat are important risk factors for obesity-related cardiac dysfunction.⁹¹ Echocardiography, CT, and magnetic resonance imaging have been used to evaluate EF, but variations between methodologies limit the comparability between these modalities.^{92–94} EF causes local inflammation and may have direct effects on coronary atherosclerosis.

Among the various adipocytokines, there has been considerable emphasis on leptin, adiponectin, and interleukin-6 (IL-6),^{95–99} which stimulate the production of C-reactive protein (CRP) by the liver. The cardioprotective effects of adiponectin are attributed to its ability to suppress apoptosis, oxidative stress, and inflammation in cardiomyocytes. It may act directly on cardiomyocytes to protect the heart from ischemic injury, hypertrophy, cardiomyopathy, and systolic dysfunction.^{100–103} These results link adipose tissue-derived factors to overt CVD in humans; CRP, fibrinogen, and IL-6 are associated with CVD and death in general populations.^{95,104} Other factors, such as proinflammatory and prothrombotic states have also been associated with metabolic syndrome.^{75,105}

Nonspecific Biochemical Enzyme and Isoenzyme Markers of CVD

The heart and vascular bed have typically been identified as the primary sites of atherosclerosis; the obesity epidemics

have revealed that adipose tissue and liver play crucial roles in the development of risk factors for CVD.

Myocardium insult or injury is accompanied by elevated serum levels of the activities or mass of AST (EC 2.6.1.1),¹⁰⁶ creatine kinase (EC 2.7.3.2), MB isoenzyme (CK-MB),¹⁰⁷ and LDH (EC 1.1.1.27) isoenzyme 1¹⁰⁸ because these enzymes are enriched in the myocardium compared with other tissues and in normal serum.^{109,110} This relationship is highly specific and sensitive for acute heart injury, notably acute MI (AMI).^{109,111} The World Health Organization recommends AST, CK, and LDH panel for the diagnosis of AMI.¹⁷ Elevated CK-MB levels, along with abnormal AST and LDH-1 was seen in some patient with chronic heart diseases, electric cardioversion for heart rhythm disturbances, coronary catheterization, and exercise.

Noncardiac pathological conditions also increase the activities of these enzymes. Skeletal muscle has low CK-MB levels, substantial injury increases CK-MB activities/mass in the blood, and reaches abnormal values.¹¹² Another condition that can mimic MI in its clinical presentation is a pulmonary embolism (PE).^{113,114} AMI can be distinguished from PE by serial enzyme analysis of AST, CK-MB, and LDH-1 in serum out every 12 hr for first 48 hr by an accurately known time of onset of symptoms. The interorgan relationships and crosstalk between liver, muscle, kidney, and lung in the development of CVD have been studied extensively in adults.

ALT and AST

ALT and AST, also known as liver transaminases, are intracellular enzymes which catalysis transamination reaction between amino acids and ketonic acid.¹⁰⁶ ALT is a widely used serum marker of the liver disease since it is predominantly found in hepatocytes. Unlike ALT, AST derived significant portions from other tissues such as heart, red blood corpuscles (RBC), and muscle; hence, it was the least desirable marker of liver function. Recent studies demonstrate the role of liver transaminases as independent predictors of cardiac-related morbidity and mortality.^{106,115–117}

AST levels in the blood increase between 3 and 4 hr after AMI, reach a peak level within 15–28 hr and return to normal range within 5 days.²⁵ AST activity increased in several other clinical conditions such as hepatic congestion secondary to congestive HF, electrical cardioversion, myocarditis, and pericarditis. However, despite its high sensitivity for use in identifying AMI, AST is a nonspecific biomarker of cardiac tissue.¹¹⁸

Two distinct isoenzymes of AST exist in human tissues: cytosolic (soluble) AST (c-AST/s-AST) and mitochondrial AST (m-AST). These isoenzymes have different immunochemical and kinetic properties and amino acid composition.¹¹⁹ Compared with c-AST, m-AST is elevated after myocardial injury,^{120,121} increases later, and provides different biological information.¹²² Also, higher m-AST isoenzyme correlated significantly with the invasive hemodynamic estimation of LV dysfunction of myocardial necrosis in a subset of 15 AMI patients.¹²¹

ALT a commonly used prediction parameter of nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes, and also few studies have been conducted to investigate the relationship between circulating ALT/AST and CHD.^{123,124} Furthermore, it has proposed that elevated circulating levels of ALT and AST are associated with endothelial dysfunction

(carotid artery intima-media thickness and flow-mediated dilatation)-induced atherosclerosis and inflammation.¹²⁵ Data from the Framingham Heart Study have shown, after adjusting for confounding variables, over a 10-year follow-up period among 3744 white adults in the United States who were free from clinical HF at baseline, there was an increased risk of AF associated with increased serum, either marker ALT or AST (>40 U/L).^{115,126} Thus, ALT and AST levels are independently associated with increased CVD-related mortality and serve as surrogate predictive markers in CVD progression.^{106,116,117,124,127}

Lactate dehydrogenase

LDH is an omnipresent enzyme that transfers a hydride from one molecule to another, which catalyzes the interconversion of lactate to pyruvate, where it converts NAD⁺ to NADH and back again. It is a tetramer enzyme protein, and the two most common subunits are the LDH-M and LDH-H. These two subunits can form five possible tetramers (isoenzymes): 4H, 4M, and the three mixed tetramers (3H1M, 2H2M, 1H3M). These tetramers are enzymatically alike; but isoforms show different tissue distribution: M4 has four muscle (M) subunits in the isoenzyme of skeletal muscle and liver, but H4 in most species, contains four heart (H) subunits, the principal isoenzyme for the heart muscle.

In AMI, the serum levels of LDH-1 are higher than LDH-2 (a “flipped pattern”) because damage to heart tissues releases the heart-specific isoenzyme into the circulation, which is rich in LDH-1, unlike normal condition LDH-2, which is the predominant circulating form.^{128,129} The ratio of LDH-1 to LDH-2 (LD1/LD2) is a well-known marker for the late diagnosis of MI^{130,131} because it provides more organ specificity than total LDH activity.¹³² The blood levels of both LDH and its isoenzyme LDH-1 are elevated 5–10 hr after AMI and reach maximum value within 60–144 hr, and return to normal range within 12 days.¹³² Diagnostic use of the LDH-1 isoenzyme is limited, however, because levels are typically elevated during acute renal failure and with hemolytic anemia.¹³³ The use of LDH to diagnose MI is superseded by the measurement of cardiac-specific Troponin I or Troponin T.¹³⁴

Creatine kinase

CK or creatine phosphokinase (CPK) also known as phosphocreatine (PCr) kinase, is a ubiquitous cytosolic enzyme. CK catalyzes the interconversion of creatine to PCr and with an expenditure of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and back again.¹³⁵ This enzyme consists of two subunits, either B (brain type) or M (muscle type), which makes three different isoenzymes: CK-MM, CK-BB, and CK-MB. Skeletal muscle expresses high levels of CK-MM (98%) compared with CK-MB (1%). In contrast, cardiomyocytes express CK-MM (70%) and CK-MB (25%–30%). Brain and smooth muscle, including vascular and uterine tissue, express CK-BB isoenzyme predominantly. In addition to these three cytosolic isoforms, the ubiquitous and the sarcomeric form of two mitochondrial CK isoenzymes are also present in tissues.¹³⁶

Clinically, the blood CK assay is used as a marker of damage to CK-rich tissue such as the damage that occurs in MI,¹³⁵ acute kidney injury, autoimmune myositides,

muscular dystrophy, and rhabdomyolysis (severe muscle breakdown).¹³⁷ CK-MB levels increase with myocardial damage.¹³⁵ The blood level of CK-MB, the bound combination of two variants (isoenzymes CK-M and CK-B) of the enzyme PCr kinase,¹³⁸ is a marker of AMI. It appears in the blood 3–9 hr after an AMI, a maximum value in the blood within 10–20 hr, and returns to normal range ~72 hr¹³²; sensitivity is high if the blood is collected during the early onset of the disease. The primary source of CK-MB is the myocardium, although skeletal muscle is another source. The relative index is quantified by the ratio of CK-MB (mass; not activity) to total CK to distinguish false-positive elevations of CK-MB from skeletal muscle.

CK-MB1 and CK-MB2 are the isoenzymes of CK-MB. CK-MB2 is the tissue form released from the myocardium after an MI¹³⁹ and rapidly converted peripherally into CK-MB1 isoform after the onset of MI symptoms. Generally, CK-MB1 a major isoform; thus, the CK-MB2/CK-MB1 ratio is typically less than 1. When CK-MB2 is elevated, and the ratio is >1.7, the result is positive. The recent improvement in measuring different isoforms of the B subunit specific to cardiomyocytes, the ratio between the CK-MB1 and CK-MB2 isoforms¹⁰⁷ makes CK measurement a more specific and sensitive test for MI.

In the case of the children, the levels of CK-MB depend on the form of cardiomyopathies. In general, increased MB fraction is associated with the myocardial dysfunction with significantly higher levels with restrictive and dilated cardiomyopathy compared with a hypertrophic cardiomyopathy in children.¹⁴⁰ Further studies of this aspect may provide validation for the use of CK-MB1 and CK-MB2 as early prognostic markers in predicting cardiac dysfunction in children with obesity or other risk factors.

Myoglobin

Myoglobin is a small globular oxygen-carrying protein found in the heart and striated skeletal muscle, with high homology sequence in both tissues.^{141,142} Levels of myo-

globin rise rapidly after AMI, and it has become a valuable cardiac biomarker in the differential diagnosis of suspected AMI¹⁴³ because plasma CK and CK-MB levels reach significant levels only 6 hr after the necrosis. During MI, the levels of myoglobin rise during the 1–3 hr of myocardial cell death after AMI, peak at 4–10 hr, and return to normal in 1–1.5 days^{132,144,145} (Fig. 5).

The level of myoglobin is higher and cleared more rapidly from plasma after coronary reperfusion or no reperfusion (thrombolytic therapy) in patients and thus allows discrimination between these two conditions.^{146,147} Myoglobin levels in serum are falsely elevated under certain conditions, such as skeletal muscle and neuromuscular disorders, renal failure, intramuscular injection, and strenuous exercise, which was unrelated to AMI.¹⁴⁸ Combining, skeletal-specific marker (carbonic anhydrase III) or a cardiac-specific marker (troponin I), as well as evaluation of successive myoglobin samples, may represent a novel strategy for myoglobin measurement that can resolve this.

Glycogen phosphorylase isoenzyme BB

Glycogen phosphorylase (GP) is an essential enzyme that regulates carbohydrate metabolism by mobilization of glycogen. It catalyzes first rate-limiting step of glycogenolysis, which converts glycogen into glucose-1-phosphate.¹⁴⁹ GP exists in three different isoenzymes for the tissues in which they were initially identified, namely GPLL (liver), GPMM (muscle), and GPBB (brain).^{149,150} Higher levels of GPBB can also be found in the heart muscle, where the 94 kD monomer is specifically present, which was comparable to other tissue concentrations.¹⁵¹

An early phase of AMI showed a rapid elevation of GPBB in the circulation.^{31,152} Hence, GPBB is ideal for early diagnosis of ischemic heart disease, with high sensitivity (0.97) and specificity (0.81) in patients with AMI within 3 hr of the onset of symptoms.¹⁵³ GPBB is found in the heart and brain; a serum increase of GPBB should be considered highly specific for myocardial injury once

FIG. 5. Comparison of cardiac biomarkers after levels and time of peak after the onset of AMI. Source: https://www.slideshare.net/anurag_yadav/cardiac-biomarker-past-today-and-future. AMI, acute myocardial infarction. Color images are available online.

damage to the brain and resulting perturbation of the blood/brain barrier has been excluded. GPBB and glycogen are tightly associated with the vesicles of the sarcoplasmic reticulum in the aerobic heart muscle. During the onset of hypoxia that heralds ischemia, tissue glycogen is broken down, and GPBB is converted from a structurally bound form (GP b) into a free soluble cytoplasmatic form (GP a), thereby accelerating glycogen breakdown.¹⁵¹ A high concentration gradient of GP in the sarcoplasmic reticulum compartment allows efflux of GP into the bloodstream through the T-tubule system.¹⁵¹ Ischemia-induced structural alterations in the cell membrane^{151,154} effluxes GPBB into the extracellular fluid. Therefore, the amount of association with the sarcoplasmic reticulum glycogenolysis complex depends primarily on the metabolic state of the myocardium.¹⁵⁵ Based on the localization of GPBB in the heart muscle and increased plasma levels of GP during ischemia and hypoxia, many studies have demonstrated that plasma GPBB is a surrogate marker of the ACS.^{151,155–158}

ST2

ST2 is a member of an IL-1 receptor family and exists in transmembrane (ST2L) and soluble (sST2) isoforms.^{37,159,160} Cardiomyocytes secrete ST2L/sST2 into the circulation, whereas pulmonary endothelium does the same in response to inflammation and cardiac diseases.^{160–162} Secreted sST2 inhibits IL-33/ST2 signaling by functioning as a decoy IL-33 receptor, thus downregulating the inflammatory response.¹⁶³ Patients with acute HF show higher levels of sST2 than in those without; N-terminal pro-brain type NP (NT-proBNP) is superior in the diagnosis of HF.¹⁶¹ High levels of sST2 correlate with the severity of disease and increased morbidity in patients with acutely decompensated HF and ACS.¹⁶³

The ST2 transcripts upregulated in mechanically stimulated cardiomyocytes. Both ST2L and sST2 were induced by HF, with the sST2 displaying a more robust expression.¹⁶⁰ Also, data from the PRAISE-2 HF trial indicate that the independence of brain-type NP (BNP) and proatrial type NP (proANP), the alterations in ST2 remained significant as an interpreter of mortality or transplantation.³⁷ Thus, serum sST2 may be a novel biomarker for neurohormonal activation in patients with HF.³⁷ Laterally, many studies have confirmed the prognostic value of sST2 in HF patients.^{161,164–166} compared with its minor value for diagnostic purposes.^{161,164,167} An advantage of sST2 compared with other NP markers is that its concentration is not affected by age, body mass index, or renal function.¹⁶⁷ Hence, sST2 seems to be a specific potential marker for therapy guides for HF, but it is less sensitive than BNP for acute HF, which requires further evaluation.^{168,169}

Ischemia-modified albumin

The conventional modern cardiac markers (CK-MB, Troponin I, and Troponin T) are more specific and represent a sensitive test for the detection of myocardial necrosis, but their levels are delayed, rising ~3–6 hr after the onset of the myocardial injury. Henceforth, it is one of the many reasons why it continues to be challenging to find diagnostic approaches for suspected clinical ACS or cardiac dysfunction. Early identification of patients with AMI is extremely critical.¹⁷⁰ Because their presentations can vary widely with

symptoms such as breathlessness, chest pain, epigastric discomfort, nausea, and vomiting may be subtle and not immediately associated with cardiac dysfunction^{170,171} and they are associated with high mortality. During acute myocardial ischemia, most of the biomarkers are negative, IMA is highly sensitive and detectable in the early reversible phase of ACS.^{171,172}

IMA is a modified form of human serum albumin (HSA) in which the N-terminal amino acids have been modified by ischemia as the result of acidosis, energy-dependent membrane disruption, free-radical injury, and hypoxia.¹⁷³ The increase in IMA levels occurs immediately after the onset of ischemia and returns to baseline values within 6–12 hr, thus enabling early identification of ischemia.¹⁷⁴ Unlike other specific and sensitive markers, the levels of IMA surged early in the onset of disease, thus aiding in the diagnosis of acute ischemia before the onset of myocardial necrosis event.¹⁷⁴

In 2003, the Food and Drug Administration (FDA) approved the albumin cobalt-binding (ACB) assay for the patients admitted to the emergency department in identifying myocardial ischemia. The binding of the cobalt enables the measurement of IMA to the damaged N-terminus of the albumin.¹⁷⁵ The N-terminus of HSA is the primary binding site for the transitional metals, cobalt and copper.^{175,176} During ischemia, the metal-binding site in HSA is susceptible to biochemical changes compared with albumin from other species. The mechanism by which it reduces metal-binding ability at its N-terminal is unknown.

It has been assumed that IMA is “FA occupied” albumin rather than “N-terminal modified” albumin in patients with acute myocardial ischemia. However, instead of measuring IMA using an enzyme-linked immunosorbent assay (ELISA), it is better to use the standard ACB assay, which deals with “FA occupied” but not actually “N-terminal modified” albumin.^{34,176,177} Highly elevated serum levels of free fatty acids (FFA) with high levels of IMA correlated positively in myocardial ischemic patients.¹⁷⁸ Thus, FFA binding alters conformational changes and inhibits the metal ion binding to albumin.^{179,180} Chronic diseases associated with “glycated/oxidized” albumin, oxidative stress generates “irreversible” oxidation-modified albumin secondary to oxidation adducts on albumin.¹⁷⁶ Henceforth, the use of both standard ACB assay and ELISA tests for determining IMA levels is ideal. Although the understanding of the IMA marker is limited, its sensitivity to and specificity for myocardial ischemia before cell damage may be an enormously valuable biomarker of prognosis. Further studies on the modification by modern proteomics tools would be interesting.

Heart Tissue-Specific Novel Biomarkers

The well-established traditional cardiovascular biomarkers (including LDL and HDL-C, glucose, and others) and many additional putative biomarkers (including MPO, pregnancy-associated plasma protein A, soluble CD40 ligand, and others) are not included in this review, because our focus is on the cardiac-specific biomarkers that show the most promise for use as clinical biomarkers.

Cardiac troponins

cTn are the machinery of the actin thin filaments of the sarcomere of striated muscle that regulates excitation/contraction

FIG. 6. The cTn complex. The troponin complex is a component of the thin filaments in striated muscle complexed to actin. There are three types of troponins: troponin T (tropomyosin binding), troponin I (inhibitory protein), and troponin C (calcium binding). The troponins are three different structurally unrelated proteins; cTnT and I differ significantly from the skeletal muscle form. cTnT, cardiac troponin T. Color images are available online.

coupling in the heart (the contractile apparatus in the myocardium).¹⁸¹ Cardiac muscle-specific isoforms cTn subunits (T=tropomyosin binding, I=inhibitory, and C=calcium binding), cardiac troponin T (cTnT), and cardiac troponin I (cTnI) expressed exclusively on the thin myofilament of the contractile apparatus, with a small proportion (3%–6%) as unbound protein in the cytoplasm of myocytes (Fig. 6).^{182,183}

Due to their high specificity and absolute cardiomyocyte specificity, cTnI and cTnT are now considered the ideal biomarkers for detecting cardiomyocyte injury,^{184,185} compared with cardiac enzymes or CK-MB mass.²⁶

Circulating levels of cTn can be indicative of myocardial injury, and it is the preferred biomarker for the diagnosis of MI in all guideline-approved algorithms in the diagnosis of ACS. Troponin elevation following myocardial necrosis starts ~2–3 hr and peaks within 24 hr, and continue for 1–2 weeks (Fig. 5).¹⁸⁶ The detection of high-sensitivity cTnI is used to diagnose MI, and high-sensitivity assays for cTnT are used to predict the risk of CHD, HF, and death.¹⁸⁷

Natriuretic peptides. NPs have attracted considerable attention as indicators of metabolic regulation in the cardiovascular system.^{188–191} NP together is called as atrial NP (ANP) or factor. B-type NP (BNP), which was called initially brain NP, and C-type NP (CNP) with the ability to relax smooth muscle, was purified from porcine brain extracts. NP family members have highly conserved amino acid sequence homology with a typical characteristic ring structure. The heart expresses all types of NPs, but BNP and ANP are regarded as principally cardiac secretory products.¹⁹² In response to stretch, myocardium releases NP and promotes natriuresis, vasodilatation, and myocardial relaxation.¹⁹³ All NPs are synthesized as preprohormones (Fig. 7A). NPs are reputable as markers of diagnosis and prognosis in HF. However, due to prognosis indicators in a wide variety of disease states as well as in apparently healthy individuals, the usefulness of NP extends far beyond patients with acute or chronic HF. Also, NP plays a central role in the regulation of body weight and energy metabolic homeostasis.¹⁹⁴

NP receptors and their actions. The actions of NP are through membrane-bound receptors, NP receptor-A (NPR-A), which binds both ANP and BNP, and NP receptor-B (NPR-B), which binds CNP. Binding of the NP to their cognate receptors activates their guanylyl cyclase intracellular domain and produces cGMP, the second messenger that elucidates their downstream actions. NP receptor-C (NPR-C), a third receptor called the NP “clearance” receptor, because it lacks an intracellular domain, binds all three NPs (ANP, BNP, and CNP) and internalizes the NP, which is then subjected to intracellular degradation.^{192,195} Possibly, NP is also degraded by peptidases in the circulation (Fig. 7B).

Atrial NP. ANP is a hormone synthesized by atrial cardiomyocytes and released in response to elevated atrial distention. Human pre-proANP is a 151 amino acid in length prohormone. N-terminal signal sequence cleavage resulted in a 126 amino acid proANP, which is the predominant stored form in atrial granules. Upon secretion, proANP is rapidly cleaved by a transmembrane cardiac serine protease called as Corin to form the biologically active C-terminal 28 amino acid peptide and the biologically inactive fragment (98 amino acid) N-terminal prohormone ANP (NT-proANP)¹⁹⁶ (Fig. 7A).

Atrial wall stretch or distension resulting from increased intravascular volume is the primary stimulus for ANP secretion.¹⁹⁷ During HF, cardiac transmural pressure may promote ANP biosynthesis in the ventricles. Hypoxia or ischemia is one of the most potent stimuli for ANP secretion.^{198,199} Congestive HF elevates 10- to 100-folds of plasma levels of ANP (healthy patients are ~20 pg/mL).¹⁹⁰ Both ANP and NT-proANP were used as markers for the diagnosis of asymptomatic LV dysfunction,²⁰⁰ and the severity of symptomatic HF was correlated with plasma ANP levels. Recently, however, measurement of BNP levels has been preferred due to its higher stability than ANP for diagnostic and prognostic use in HF.²⁰¹

The proANP (126 amino acid), has a longer half-life and makes serum measurement more feasible; a novel assay that

A Tissue Expression

Proteolytic processing of pre-pronatriuretic peptides

Mature Natriuretic peptides

B Natriuretic peptide Receptors and its degradation

FIG. 7. Cardiac NPs system. (A) Tissue expression of NP, proteolytic processing of pre-pro-NPs to mature and biologically active peptides. (B) NP-binding receptors, intracellular signaling, and degradation processes. ANP, atrial natriuretic peptide; BNP, brain-type natriuretic peptide; cGMP, cyclic guanosine monophosphate; CNP, C-type natriuretic peptide; GC-A, guanylate cyclase type A; GC-B, guanylate cyclase type B; GTP, guanosine triphosphate; IDE, insulin-degrading enzyme; NEP, neprilysin; NP, natriuretic peptide; NPR, natriuretic peptide receptor. Color images are available online.

detects the mid-regional zone of proANP (MR-proANP) is now available and has been evaluated as a test for HF.²⁰² In two studies, the Biomarkers in Acute HF (BACH, 1641 patients) trial²⁰³ and the PRIDE study²⁰⁴ show that measurements of MR-proANP improve the diagnostic accuracy of BNP gray zone (levels of BNP ~ 100 – 500 pg/mL) in obese patients. Hence, the combined use of MR-proANP and either BNP or NT-proBNP provides superior diagnostic precision compared with the use of either alone.^{205,206}

B-type NP. Cardiomyocyte-specific BNP is synthesized as a prehormone of 134 residues, containing a signal sequence that is cleaved to yield a 108 amino acid prohormone. The 108 amino acid precursor, proBNP, is produced in the cardiomyocytes, the biologically active C-terminal 32 amino acid BNP (BNP 1–32) and the inactive 76 amino acid N-terminal peptide [NT-proBNP] then processed by enzymes corin or furin by proteolytic cleavage between residues 76 and 77. Therefore, the heart secretes all three peptides; human BNP is 32 amino acids long but fully processed BNP length varies among species^{191,207}; (Fig. 7A).

Currently, there are no specific data to support the use of any specific biomarkers as routine preclinical indices of CVD in obese children. BNP and cTn have gained importance as biomarkers in CVD. In adults, a relationship has been observed between plasma BNP/NT-proBNP or cTn levels in clinical CVD severity, particularly in HF. Also, it has been shown that NP plays a central role in the regulation of body weight and energy metabolism.¹⁹⁴ Interestingly, the previous study shows that BNP levels are significantly lower in overweight and obese adult subjects than in those of healthy weight,²⁰⁸ probably contributing to insulin resistance and hypertension.

BNP inhibits renin and aldosterone secretion by decreasing sodium retention and increases the glomerular filtration rate.²⁰⁹ Healthy individuals have circulating plasma BNP concentrations of around 1 fmol/mL (3.5 pg/mL). In case of patients with congestive HF, plasma BNP concentrations, as well as those of the inactive precursor NT-proBNP, have been shown to increase up to 100-fold.²⁰⁰ NT-proBNP concentrations in healthy individuals are ~ 51 pg/mL; the cutoff levels are 300 pg/mL for a diagnosis of acute HF and 125 pg/mL for chronic HF. For BNP, the cutoff concentrations for the diagnosis of acute and chronic HF are 100 and 35 pg/mL, respectively.²¹⁰ The normal ranges and cutoff points of the two markers are different because concentrations of NT-proBNP are substantially higher than those of BNP due to a longer half-life. Tests for both of these peptides are commonly used as markers of prognosis in chronic HF condition and to rule out the diagnosis of HF. Both BNP and NT-proBNP are established markers of HF; however, their measurements are not interchangeable. The BNP/NT-proBNP is also used as a biomarker of HF in children. Even though there is a steep age-dependent variation in healthy individuals, concentrations are ~ 3000 pg/mL in infants 0–2 days of age compared with concentrations of ~ 100 pg/mL for children between 1 month and 1 year of age.^{211–213}

NT-proBNP is unable to activate NPR-A and generate cGMP, and the ability of proBNP to do so is markedly reduced. This reduced activation of NPR-A by proBNP and NT-proBNP is highly relevant to the pathophysiology of HF as increasing evidence demonstrates that proBNP and not BNP 1–32 is the predominant circulating form.²¹⁴ BNP 1–32

undergoes further processing by dipeptidyl peptidase IV, which removes the two N-terminal amino acids (Ser and Pro), producing BNP 3–32.²¹⁵ Importantly, BNP 3–32 is also in the circulation, and its concentrations increase in human HF.²¹⁶ Its physiological significance is high since BNP 3–32 as compared with BNP 1–32 has reduced natriuretic and diuretic properties and lacks renal vasodilatation.²¹⁷

Sensitive MS methods have identified many new circulating forms of truncated, low molecular mass proBNP, and NT-proBNP. The proBNP (3–108) peptide circulates at relatively low concentrations in healthy subjects, but in patients with asymptomatic LV dysfunction or HF concentrations of proBNP are elevated several folds. Circulating forms of proBNP are produced by the action of either circulating or cell surface enzymes, such as neprilysin, dipeptidyl peptidase IV, insulin-degrading enzyme, and meprin (Fig. 7B).¹⁹² Thus, chronic HF represents a state of relative BNP deficiency.

HF may represent a deficient state of biologically active NP. The abnormality in the processing of NP, leading to a deficiency of mature BNP conversely elevated levels of biologically inactive BNP detected in chronic HF.²¹⁸ Also, myocardial NEP mRNA levels elevated in the patients with HF, leading to accelerated degradation of NP, supports the hypothesis that a deficiency of NP may be present in HF²¹⁹ and may be due to abnormal processing or accelerated degradation. At the same time, the glycosylation and truncation of these peptides offer considerable scope for new BNP form-specific assays that may better reflect the presence, progression, or prediction of HF and its outcome.

C-type NP. CNP complements the endocrine actions of ANP and BNP by serving as a paracrine component of the NP axis. CNP is the widely expressed NP in the brain and chondrocytes, and cytokine-exposed endothelial cells secrete highest concentrations.^{220–222} In humans, the primary source of circulating CNP are endothelial cells and heart.²²² Hence, it appears to play a significant role in vascular and cardiac function, both physiologically and pathologically. Both the endogenous and exogenous CNP may offer protection from ischemia/reperfusion injury. Mice overexpressing CNP in cardiomyocytes show reduced pathologies such as ventricular hypertrophy, necrosis, inflammation, and functional impairment of cardiac tissue induced by coronary artery ligation^{223,224} (Fig. 7A).

Human proCNP 103 residues are processed to produce the mature 53 amino acid peptide by an intracellular endoprotease, Furin,²²⁵ which is the predominant form in the brain,²²⁶ endothelial cells,²²⁷ and heart.²²⁸ CNP-22 is the cleaved peptide from CNP-53 by an unknown extracellular enzyme; both elicit similar, if not, identical functions,²²⁹ but their tissue expression differs (Fig. 7A). CNP does not behave as an exclusive cardiac-specific hormone, although its potential role in HF cannot be ruled out.¹⁹¹ Elevated in patients plasma CNP concentrations (CNP-53/22 form) with congestive HF compared with the healthy subjects are in the low fmol/mL range.²³⁰

Cardiac myosin light chain kinase

The cardiac myosin light chain kinase (cMLCK) is expressed only in the heart, in both the atria and ventricles.^{21,23} cMLCK plays an essential role in maintaining the phosphorylation levels of regulatory MLC2, a cardiac isoform of

the regulatory light chain thought to be crucial for the regulation of cardiac function. cMLCK was identified in the heart^{21,231} and has been reported to be an essential kinase for MLC2 phosphorylation.^{21,231,232} In the myocardium, cMLCK is responsible for the basal phosphorylation of both MLC2v and MLC2a *in vivo*²³³ and alters structural changes in both thick and thin filaments that enhance contraction of heart muscle.²³⁴ The contractile dysfunction, ventricular hypertrophy, necrosis, and fibrosis was seen in the selective abolishment of MLC2v phosphorylation.^{211,233,235,236} cMLCK was identified in human HF,²¹ where it may play an essential role in adaptive responses to pathophysiological stresses in the heart.^{21,233,235–237}

A reduction in phosphorylation of MLC2 was demonstrated in adult patients with HF.^{238–240} *Myh3* gene that encodes cMLCK was ablated either from the germline or inducibly in adulthood; both populations of cMLCK-deficient mice exhibited HF.^{237,241} To the best of our knowledge, it remains unknown whether phosphorylation of MLC2 or its active kinase, cMLCK, is reduced in children with HF.

Heart-type fatty acid-binding protein

FABP are a family of cytoplasmic or intracellular fatty acid transporters.²⁴² There are brain-type FABP (B-FABP), intestinal-type FABP (I-FABP), liver-type FABP (L-FABP), and H-FABP^{242,243} evolved by successive gene duplications generating a large number of tissue-specific homologs. Higher rates of FA uptake and lipid metabolism tissues have high expression of one or more FABPs. This vast diversity of FABPs, which contrasts sharply with other lipid-binding proteins, has long been suggestive of functional specialization.²⁴²

Since H-FABP exists mainly in cardiomyocytes, it becomes a potentially promising biomarker for myocardial injury.^{36,244} FABP family members involved in active fatty acid metabolism, functionally H-FABP also do the same, where it transports FAs from the cell membrane to mitochondria for oxidation.²⁴⁵ This FABP are believed to participate in the uptake, intracellular metabolism, and transport of long-chain polyunsaturated fatty acids. H-FABP is rapidly released into the cytosol early in AMI.²⁴⁵

H-FABP represents 60%, which is significantly higher and superior sensitivity than that of cTnI (18.8%) and CK-MB (12.5%) and adds incremental value to troponin in early diagnosis of an ACS (within 5 hr of the onset of symptoms).^{36,246–250} However, the specificity was only 23.53%, which is less than that of CK-MB (100%) and cTnI (66.67%). During the period from 4 to 12 hr of the onset of symptoms, the sensitivity of H-FABP was 86.96%, which was comparable to cTnI and CK-MB without compromising specificity.^{36,246–250}

The mechanism of H-FABP leakage in a failing heart has been elucidated in a few animal studies. Insulin-like growth factor 1 signaling inhibits microRNA-1 expression and plays a critical role in cardiac hypertrophy, which results in a release of H-FABP from myocardial tissues to circulation.²⁵¹ During HF, the energy substrate used switches from reduced FA oxidation to increased glucose utilization, which differs from the cardiac metabolic process in a healthy heart.^{252–255} It was shown that in H-FABP knockout mice there was depressed cellular uptake of long-chain FA and its oxidation, indicating the critical role of H-FABP cardio-

myocytes.²⁵⁵ Furthermore, extracellular circulating A-FABP and H-FABP suppress cardiomyocyte contraction by reduced excitation/contraction coupling through reduced intracellular transient calcium.²⁵⁶ In obese subjects, adipocytes FABP4 may be involved in the initiation or progression of cardiac contractile dysfunction. Through the alteration of cardiac energy metabolism and cardiac dysfunction, H-FABP leakage from damaged myocardium in the failing heart may contribute to a vicious cycle that finally leads to poor clinical outcomes.

Additionally, an H-FABP level increased in association with more significant number of cardiovascular risk factors and was an independent risk factor for all causes of cardiovascular death. It is noteworthy that circulating H-FABP concentrations are elevated in NAFLD and metabolic syndrome as well as in obesity,^{257–261} signifying that H-FABP is one of the markers of insulin resistance and subclinical myocardial damage that exist in these patients. For this reason, the diagnostic accuracy of H-FABP may make it a useful indicator for the early prediction of high-risk patients in the general population,^{244,262} as well as being considered a useful marker for assessing ongoing myocardial damage.

Noninvasive Anatomical and Electrophysiological Cardiac Biomarkers

The commonly used CVD surrogate biomarkers include (1) Soluble biomarkers (lipids and inflammatory markers), acute-phase proteins, and adipokines; (2) Physiological measures (blood pressure, endothelial function, and vascular stiffness); and (3) Anatomical measures [CIMT and CAC]. The measurement of CVD biomarkers at multiple points along the pathophysiological pathway as indicators of disease traits, disease state, the rate of progression, or as surrogate endpoints for treatment trials are useful.¹⁶ Also, a growing body of research suggests that noninvasive measures of vascular morphology and functions may predict pediatric cardiometabolic risk factors' early presence of surrogate markers must predict later onset of CVD.^{263,264} Also, the limitations of surrogate markers such as involved in the multipathway process, are not involved in diseases and inappropriately sensitive or responsive to intervention, and often lack methodological standardization in children.²⁶⁴

Carotid intima-media thickness

The risk of future CVD events, including MI and death correlates with CIMT ultrasound measurements.^{265–267} Absolute quantitative measurements of the CIMT and its change over time is assessed by advanced B-mode ultrasound imaging techniques.²⁶⁸ CIMT measurement is an adequate alternative assessment for atherosclerosis (arterial system) and CV events (a surrogate endpoint) in intervention studies.^{263,268,269} The rich dataset includes 23.4 years of longitudinal follow-up on 2893 twelve- to 18-year-old participants from four studies from the International Childhood Cardiovascular Cohort (i3C). Data collected across three continents show that childhood obesity, hypertension, and dyslipidemia in adolescence predict the presence of high CIMT in adulthood.⁷³ Thus, the clinical cardiovascular endpoints of atherosclerosis can be assessed by standard CIMT measurement in younger populations.⁶⁹

Coronary artery calcification

Patients with a high prevalence of CHD show CAC and are associated with significant adverse cardiovascular events.^{270–272} There are two common types of CAC, intimal and medial calcification, each of which has specific risk factors. Medial calcification is associated with arterial stiffness, which increases the risk of adverse cardiovascular events.²⁷¹ The foremost noninvasive tool used to detect calcified lesions is coronary CT angiography.²⁷⁰ Since adiposity is the primary determining factor of CAC in obese youth and adults, CAC is the independent risk factor used to predict subclinical atherosclerosis.^{273,274}

Dysfunction of the autonomic nervous system and HRV

The autonomic nervous system (ANS) has an essential influence on the progression of CVD. The two halves of the ANS, the parasympathetic nervous system (PNS) and the SNS, cooperate to control heart rate, cardiac output, myocardial contractility, and constriction and dilatation of blood vessels.^{275,276} Dysfunction of the ANS contributes to the development or stabilization of obesity and is associated with cardiovascular mortality.²⁷⁷ The study of ANS function in obesity is of considerable clinical interest. Methods used to assess ANS function outside the cardiac system include sympathetic skin response and quantitative pupillography.²⁷⁸ The cardiac autonomic modulation of HRV analysis has a direct association with cardiovascular prognosis.²⁷⁹ The lower the HRV, the higher the chance that coronary events will occur.²⁸⁰ Additionally, the dysfunction of autonomic cardiac control has been described among the pathophysiologic characteristics of childhood obesity.^{281–283} Among obese children and adolescents, reductions in HRV and vagal modulation have been observed during the increase of the sympathovagal balance.^{281,282,284}

Cardiac autonomic dysfunction is a risk factor for the development of CVD that can be measured noninvasively using HRV.²⁸⁵ HRV is thought to be the result of an adaptive response in heart rate caused by the SNS and PNS working together to buffer blood pressure.²⁸⁶ Altered compensatory change (low HRV) suggests a decreased adaptive ANS and is associated with increased morbidity. HRV is associated with morbidity and mortality after MI.^{287–289} AML, cardiac death, congestive HF, and arrhythmias have also been associated with reduced HRV.^{290–293}

Two different methods of electrocardiogram (ECG) are employed to assess the cardiac autonomic nerve functions, temporary differences between continuous ventricular depolarization consist of three waves QRS complexes (R–R intervals) and the spectral analysis of the ECG.²⁹⁴ This has been validated and has demonstrated good reproducibility, high sensitivity, and specificity is the analysis of time-domain measures of HRV.²⁹⁵ For accurate determination of HRV, standardized conditions are necessary, since it can be influenced by many factors, including age, heart and respiratory rate, blood pressure, food ingestion, coffee consumption, smoking, body position, volume status, mental stress, drugs, exercise, and time of day.²⁹⁶ However, the majority of studies that assessed HRV in obese children and adolescents showed significantly higher blood pressures than their nonobese peers.^{281,283} There is a direct association between high arterial blood pressure and the impairment of cardiac autonomic modulation.^{297,298} Hence, this change in the hemodynamic characteristic may itself be an independent cause of the cardiac autonomic dysfunction observed in this population.

MicroRNAs and Exosomal miRNAs Are Gene Regulators and Emerging Biomarkers with Therapeutic Potential

MicroRNAs are involved in the developmental processes in vertebrates. Apart from that, the role of specific miRNAs in certain disease conditions may provide insight into disease pathogenesis and potential mechanisms of repair.^{299,300} Exchange of information between organs may occur through exosomes; since it has been identified in body fluids and blood, they are made exosomes and their specific cargo potential biomarkers.³⁰¹ Exosomal miRNAs may reflect disease-specific aberrations. Additionally, exosomes may deliver the contents (proteins, mRNAs, miRNAs, and DNAs) to target specific cells that contain cell-specific factors that allow the exosomes to deliver the exosomal contents to recipient cells with a high degree of efficiency. Research on CVD with some exosome cargos (miRNAs, lipids, and proteins) provide the development of noninvasive discovery of novel biomarkers.^{299,300,302,303} However, their clinical use remains juvenile. A study, utilizing genetically modified mice, shows the critical requirement for miRNAs in cardiac development, as mice deficient in Dicer experience cardiac failure.³⁰⁴ In patients with CVDs, the circulating exosome-derived miR-133a levels are elevated from injured myocardium.³⁰⁵ miR-133a is implicated in the

FIG. 8. Diagnosis and prognosis miRNAs biomarkers associated with cardiac arrhythmia, AMI, and HF. miRNAs in drop-down boxes are associated with a single respective pathology, while those in *blue* and *green* boxes represent multiple corresponding pathologies. ↑; increased and ↓; decreased in the specific pathology conditions. HF, heart failure. Color images are available online.

regulation of NFATc4, a protein that contributes to cardiac hypertrophy.³⁰⁶ Furthermore, treatment of miR-133a inhibitors reduces the level of cardiac hypertrophy.³⁰⁶

The levels of miR-22, 99a, 214, and 21 increased expression or decreased expression of miR-15, 320, 132, 212, 652, 92a, and 34, which provides beneficial or protective effects in the treatment of CVD^{299,302,303} (Fig. 8). miRNAs regulate numerous biological processes, and many human diseases influence their levels of expression. Numerous challenges need to be addressed to identify miRNAs that can be reliably used as diagnostic markers or therapeutic targets for CVD and the critical role of miRNA in cardiovascular biological function.

Current Challenges and Clinical Perspective of Cardiac Biomarkers

Noninvasive measurement of end-organ function has advanced considerably with the development of cardiovascular imaging techniques, and the detection of preclinical CVD in children and youth has become more feasible.²⁶³ Depending on the marker, it can take between 2 and 24 hr for the level to increase in the blood. Additionally, determining the levels of cardiac markers in the laboratory—like many other laboratory measurements—takes substantial time. Cardiac markers are therefore not useful in diagnosing MI in the acute phase. The clinical presentation and results from an ECG are more appropriate in the acute situation. Nevertheless, long-term studies in children assessing the relationship between preclinical CVD and novel biomarkers/risk factors and its biology of the action with multiplexed detection panel are only emerging.

Conclusion

Childhood obesity has reached epidemic proportions worldwide. Several epidemiological studies identified childhood obesity and CVD risk markers in childhood as risk factors for CVD in adulthood.^{307–310} These risk factors underlie future microvascular and macrovascular disease events. Taken together, the occurrence and development of CVD are related and caused by multiple factors. Classic risk factors of CVD include type 2 diabetes, hypertension, dyslipidemia, history of smoking, and family history of CVD. Management of risk factors achieved by lifestyle changes such as sensible diet, exercise, controlling the intake of cholesterol and fat, intake of more fruits and vegetables, smoking cessation, drinking cessation, and reduction in passive smoking are exceedingly helpful in preventing CVD.

The U.S. National Institutes of Health/FDA in 2001 defined a biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.³¹¹ The levels of an ideal biomarker should be measurable in a sample such as blood, urine, or tissue biopsy and should relate to a clinical phenotype either causally or indirectly to measure the status of the diseases. The understanding of invasive and noninvasive cardiac-specific biomarkers and the risk factors may yield valuable insights into the pathophysiology and prevention of CVD in a high-risk obese population at an early stage.

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