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Review Article

Multifaceted role of cardiovascular biomarkers

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ABSTRACT

Cardiovascular diseases, a global health issue, claim the lives of many every year. Lifestyle changes and genetic predisposition are the key drivers for the development of CVDs. In many of the patients, the disease is detected at the end stage making heart transplantation the only treatment option. Hence every attempt should be made to identify the risk at an early stage and initiate preventive measures to improve the quality of their life. Biomarkers are one of the critical factors that aid in the early diagnosis of CVDs. More specific and highly sensitive biomarkers have been discovered lately and have been employed for prognosis and diagnosis of CVDs. The present review briefs about the various categories of cardiovascular biomarkers with emphasis on novel biomarkers and discusses the biomarkers employed for different purposes in CVDs. The biomarkers have also helped in identifying COVID-19 patients with increased risk for developing cardiovascular complications. Being non-invasive makes biomarkers advantageous over other methods for evaluating the pathophysiological status of CVDs.

1. Introduction

Cardiovascular diseases, a global health issue, claim the lives of many every year. Lifestyle changes and genetic predisposition are the key drivers for the development of CVDs and associated complications. CVD is a complex disease involving many pathophysiological processes. Physical signs and medical history assist clinicians in predicting the development of CVDs. However, this is possible only when the individuals experience difficulties due to underlying symptoms. In many of the patients, the disease is detected only at the end stage leaving them with little time and heart transplantation, the only treatment option. Hence attempts were made to identify the risk for CVDs at an early stage. One such is the discovery of biomarkers that has helped in identifying CVDs in the initial stages, facilitated in starting treatment strategies earlier, improving the quality of life, and expanding the lifespan.

A study in 1954 found higher levels of blood aspartate aminotransferase within 3–4 h following acute myocardial infarction (AMI). This was the first recorded cardiac biomarker.[1] Another study next year found that the levels of lactate dehydrogenase was high in patients following AMI. The levels of Creatine kinase were also higher within 72 h and were found to be more sensitive than aspartate aminotransferase or lactate dehydrogenase.[2] Since these enzymes are also present in the skeletal muscle other than cardiac muscle, they are not specific. Later, cardiac troponins were identified as a specific cardiac biomarker.[3] Since then, several different biomarkers have been recognized for use in assessing various stages of cardiovascular pathologies and distinguishing or stratifying cardiovascular patients in the first place.

Commonly used biomarkers for the detection of CVDs include natriuretic peptides (BNP and pro-BNP), blood glucose (GLU), low-density lipoprotein cholesterol (LDL-c), thyroid-stimulating hormone (TSH), high-density lipoprotein cholesterol (HDL-c), C-reactive protein (CRP), and Homocysteine (Hcy). The available and widely used biomarkers had been applied in clinical use following large clinical studies and is explained in Table 1. The drawback of many of the presently employed cardiac biomarkers is that they are affected by non-cardiac factors. Recent studies are putting efforts into adding more specific and effective biomarkers into the list and the prospective of employing them clinically. The various biomarkers and their usage in different settings of CVDs are discussed hereafter.

1.1. Metabolic biomarkers and CVDs

Metabolites are small organic molecules and include peptides, oligonucleotides, sugars, nucleosides, organic acids, ketones, aldehydes, amines, amino acids, lipids, and steroids.[4] Metabolites can also be formed in response to external sources like medications, toxins, drugs, or pathological conditions. The adverse events during cardiovascular pathophysiology affect the level of metabolites

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Table 1
List of clinical trials that tested biomarkers.

Biomarkers	Clinical Trials	Findings
NT-proBNP	PARADIGM-HF, TRANSITION, MOLITOR	Assess the risk of re-hospitalization and CV death. Quality of life.
Cardiac troponins	CORONA, RELAX-HF	Prognosis of CV death and hospitalizations.
Heart Type Fatty Acid Binding Protein	MANPRO	Predict the mortality and HF-related hospitalizations.
Galectin 3	HF-ACTION	Predictive of long-term outcomes
Soluble suppression of tumorigenicity 2 (sST2)	ASCEND-HF	Adverse HF outcomes and death

which can be utilized as biomarkers.[5] The metabolites can be easily detected in body fluids like blood, urine, and saliva. The vast list of metabolites provides an excellent database to discover newer metabolic biomarkers for CVDs, which could serve in early detection, targeted therapy and offer prognostic insights.

Nitric oxide (NO) is a metabolite that helps in diastolic relaxation and vasodilation.[6] Inflammation and oxidative stress could alter the NO synthesis from its precursor L-Arginine and hence can serve as an indicator to predict myocardial pathology. Arginine methylation due to epigenetic modifications can also alter nitric oxide synthesis. In a single-center study of chronic systolic heart failure patients with LVEF $\leq$35%, the level of pro-BNP correlated to all three arginine methylation products like asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and N-mono-methylarginine (MMA). Additionally, in patients treated with beta-blocker, ADMA and MMA levels were lower, indicating a response to therapy.[7] Another study by Shao et al, also found that the levels of ADMA were higher in the acute decompensated population.[8] These studies suggest the significance of nitric oxide regulation, arginine bioavailability, and arginine methylation in the pathophysiology of cardiac dysfunction and how such metabolites could serve as biomarkers.

Another major metabolic change observed in the failing myocardium is the switch from fatty acid to glucose metabolism. This metabolic switch leads to the accumulation of Long-chain Acylcarnitines (LCAC), the primary lipid substrates.[9] A study in 453 patients with chronic systolic heart failure along with 41 end-stage heart failure patients undergoing Left Ventricular Assist Device (LVAD) implantation observed that the LCAC levels were associated with an increased risk of cardiovascular disease and mortality. Additionally, in patients after placement of LVAD, the levels of LCAC decreased, suggesting the response to therapy.[10] A study using patients with HFpEF and HFrEF found higher levels of LCAC was associated with worse functional status and higher mortality.[11] Therefore, LCAC could be utilized as a prognostic marker for chronic systolic heart failure.

Plasma trimethylamine n-oxide (TMAO) is hypothesized to arise from the metabolism of choline by the gut microbiome and has been strongly associated with atherosclerosis and adverse cardiac events.[12] Utilizing TMAO as a biomarker could also predict the development of diseases like renal dysfunction in heart failure patients. For instance, a study tried to investigate the use of TMAO for the evaluation of HFpEF and renal function. Though patients with HFpEF have normal or near-normal ejection fractions, they exhibit cardiac associated clinical features. These patients can develop comorbidities like renal dysfunction and diabetes mellitus.[13] TMAO has been reported in patients and mice models of atherosclerosis. TMAO identified and differentiated HFpEF patients that could have renal dysfunction and predicted the risk of mortality. .[14] These patients exhibited a poor glomerular filtration rate and elevated HbA1c levels. Another study done in stable chronic systolic heart failure patients found that levels of TMAO correlated with BNP.[15] Though the precise mechanism by which TMAO contributes to CVDs is unknown, the involvement of gut-microbiome in contributing to myocardial pathogenesis is well appreciated and hence could serve as potential diagnostic biomarkers and therapeutic targets.

One of the commonly distributed natural carbohydrates, N-acetylneuraminic acid (Neu5Ac) is a functional metabolite implicated in coronary artery disease. Neu5Ac is a basic component of many glycoproteins, glycolipids, and glycopeptides and is found to accelerate thrombosis formation and promote inflammatory response.[16] Pharmacological inhibition of Neu5Ac signaling improved myocardial ischemia. A study in 766 patients with different cardiovascular pathologies found that the circulating Neu5Ac levels were significantly higher in plasma suggesting that this metabolite could serve as a biomarker for myocardial injury and could also reflect the severity of the coronary lesions.[17]

1.2. Inflammatory related cardiac biomarkers

Cardiovascular diseases are linked with inflammatory reactions and hence employing inflammatory mediators could become a reliable biomarker.[18] C-reactive protein (CRP) is one of the best-studied inflammatory biomarkers in coronary artery diseases (CADs).[19] Clinical trials like PROVE-IT, REVERSAL, AFCAPS/TexCAPS, and JUPITER found that administering statins based on CRP levels improved the cardiovascular events.[20–22] The levels of hs-CRP were associated with recurrent events in CADs. In the trial CARE, patients with elevated CRP had a higher rate of recurring coronary events.[23]

Another important protein implicated in inflammation is cytokines and include IL-1, IL-6, IL-10, tumor necrosis factor-alpha (TNF-α), and Monocyte Chemoattractant Protein-1 (MCP-1). IL-1 and IL-6 drive the production of CRP and IL-6 causes plaque instability by inducing the expression of matrix metalloproteinases (MMPs), TNF, and MCP-1.[24] In the Fast Revascularization during InStability in Coronary artery disease (FRISC-II) study, increased IL-6 levels were associated with high mortality and were independent of troponin and hs-CRP levels.[25]

In the study c7E3 Fab AntiPlatelet Therapy in Unstable Refractory angina (CAPTURE), patients with elevated IL-10 levels exhibited a decreased risk of death or non-fatal MI.[26] This data suggested that IL-10 may be protective against proinflammatory mediators in Acute Coronary Syndrome (ACS). But, another study using 158 consecutive patients with stable CAD observed that only IL-8 was a predictor of cardiovascular events.[27] Hence, the prediction of CVDs using cytokines is rather controversial and inconclusive and hence more extensive studies are warranted.

Another pro-inflammatory marker, Soluble CD40 ligand (sCD40L) has been shown to promote atherosclerosis and plaque instability.[28] High levels of sCD40L in healthy women were associated with an increased risk of cardiovascular events. Two studies, CAPTURE and OPUS-TIMI 16 concluded elevated levels of sCD40L is

an independent risk marker of cardiovascular events.[26,29] These findings imply the use of sCD40L as both biomarker and therapeutic target.

A protein associated with inflammation, calprotectin, was identified as a biomarker for the no-reflow phenomenon in Acute coronary syndrome (ACS) patients.[30] No-reflow can occur in percutaneous coronary interventions (PCI) and have very bad clinical outcomes. Early detection of no-reflow can therefore help prevent mortality and heart failure following PCI. A heterodimer of proteins S100A8 and S100A9, Calprotectin levels increased in patients with atherosclerotic plaques.[31] The higher levels of plasma calprotectin can signify a higher risk of cardiovascular deaths in ACS patients. Platelet reactivity and activation are found with no-reflow in PCI and their activation is dependent on circulating calprotectin. Though platelet activation marker (PMA) can also predict no-reflow, plasma calprotectin is an independent predictor for no-reflow.

The level of certain inflammatory biomarkers could also predict the seriousness of coronary artery disease and a good example is lipoprotein-associated phospholipase A2 (LP-PLA2) in serum. The LP-PLA2 is produced by inflammatory cells in atherosclerotic plaques and circulated in the bloodstream.[32] Studies have related the levels of LP-PLA2 with endothelial dysfunction and coronary atherosclerosis. The activity of LP-PLA2 is positively related to the number of diseased coronary branches. This biomarker could not only predict the occurrence of CAD but also predict the severity of CAD.[33] Interestingly, the inhibitor of LP-PLA2 could ameliorate the development of cardiovascular events. Fibrinogen, a clotting factor, secreted by the liver, and its circulating levels increase during acute inflammation. It is involved in endothelial dysfunction and the formation of thrombus. A large prospective study found that the levels of fibrinogen positively correlated with the incidence of cardiovascular disease.[34]

Foam cells in atherosclerotic plaque produce another inflammatory biomarker, visfatin. An adipokine, visfatin also called as Pre-B cell colony enhancing factor or nicotinamide phoribosyl transferase is implicated in the pathophysiology of obesity, metabolic disease, diabetes, and cancer.[35] The levels of visfatin correlated with the adverse cardiovascular events in acute myocardial infarction patients making it a promising biomarker.[36]

1.3. Miscellaneous biomarkers and CVDs

Labeling a specific marker for a particular cardiovascular disease would be a better approach in developing reliable biomarkers for CVDs. For instance, a study by Chen et al, noted levels of Ischemia Modified Albumin (IMA) increased during ischemia-reperfusion injury.[37] IMA, a biomarker for myocardial ischemia, can be detected relatively early and is highly sensitive. This could serve as a biomarker in patients with the chronic total occlusive condition to predict coronary collateral circulation (CCC). The higher levels of IMA predicted good CCC formation in such patients.

Biomarkers were identified that could predict the risk following surgery in cardiac patients. For instance, Out-of-Hospital Cardiac Arrest (OHCA) is a major cause of death in heart failure patients.[38] Hence finding a prognostic biomarker will aid in the early and precise detection of patients who are prone to develop OHCA. Early biomarkers of AMI like high sensitivity Cardiac troponin (hs-cTnT) and copeptin serve as prognostic indicators following an acute coronary syndrome (ACS) and NT-proBNP as a diagnostic marker of heart failure. A study in 114 patients in Norway tried to understand whether these 3 biomarkers could be used as prognostic tools to understand the OHCA.[39] Eventhough, the 3 biomarkers hs-cTnT, copeptin, and NT-proBNP could not effectively predict OHCA, it was observed that early measurement of NT-proBNP could predict one-year mortality and help in identifying patients with heart insufficiency and coronary artery diseases.

Studies have identified markers associated with several chronic and acute diseases and their relationship with the prediction of developing CVDs. For instance, the mortality observed in rheumatoid arthritis (RA) patients is majorly due to CVDs and this can be attributed to the presence of CVD risk factors in these populations.[40] Additionally, the inflammatory cytokine-induced endothelial dysfunction occurring in RA can favor the formation of atherosclerotic plaques.[41] RA-specific autoantibodies like anti-citrullinated protein antibodies formed during RA can activate inflammatory cascade. Since the myocardial interstitium of RA patients has high levels of citrullinated proteins, it can result in diastolic and systolic dysfunctions. The study found that anti-modified citrullinated vimentin levels were inversely associated with ventricular dysfunction.[42]

Certain biomarkers could predict functional impairment in heart failure patients. Since HF affects the older population, it is critical and significant to understand the effects on their day-to-day activities. For instance, the study by Niu et al, found that serum uric acid (UA) could predict the cognitive function in patients with chronic heart failure.[43] Earlier studies on the association of serum UA and cognitive function have conflicting results. This was the first study to link the association of serum UA in heart failure patients with their cognitive function. The study found that higher levels of serum UA are independently associated with poorer cognitive function in CHF patients, majorly in male patients, after normalizing for confounding variables like demographic, medical, and psychological characteristics.

Yao et al, investigated the efficiency of using multiple biomarkers for the prediction of CVDs and used CVD and non-CVD patients in the study.[44] Positive association of the serum biomarkers like FT4, TG, and LDL-c with risk of CVD was observed both in single and multiple serum biomarker models. The multi-biomarker model had much higher sensitivity and better predictivity than a single biomarker model for the prediction of CVD.

1.4. Cardiac biomarkers – risk identification

Pregnancy-associated plasma protein-A (PAPP-A) is a matrix metalloproteinase (MMP) normally produced in the placenta. . It activates insulin-like growth factor (IGF) and induces inflammation and lipid uptake contributing to atherogenesis and plaque instability. Clinical studies observed that stable and unstable CAD patients with high levels of PAPP-A exhibited increased risk for the occurrence of cardiovascular events.[45] Hence PAPP-A could be a reliable serum biomarker for risk stratification.

Another molecule that could activate MMPs and could act as a cardiac biomarker is myeloperoxidase (MPO).[46] MPO is secreted by macrophages and contributes to the formation and rupture of plaques. They induce cholesterol efflux and induce LDL oxidation. However, contrasting results have been found in clinical trials and hence could not be used as a reliable biomarker. But still, it could be employed for risk stratification in the early phase from the onset of chest pain.

Some MMPs contribute to plaque rupture. They are secreted by inflammatory cells as zymogens and activated by proteinases. MMP2, 9, and 8 are the prominent MMPs whose levels are elevated in carotid plaques.[47] The elevated levels of these specific MMPs can be used as a predictor for adverse cardiovascular events. Copeptin, a glycosylated 39-amino-acid peptide, is a biomarker of neurohormonal activation. The study found that this molecule could predict the development of cardiovascular diseases and cardiovascular-related mortality in diabetic as well as non-diabetic patients.[48] Another molecule which is also a biomarker of neural hormonal

activation, Mid-regional-pro-adrenomedullin (MR-proADM) was also found to be a potential risk predictor.[49] Study in patients found that MR-proADM is a better biomarker than NT-proBNP in predicting mortality and morbidity following HF.

Another biomolecule secreted by activated macrophages and is involved in atherogenesis is galectin 3. If patients referred for coronary angiography have high levels of plasma galectin 3 predicted risks for cardiovascular death.[50] Since heart failure patients have elevated levels of galectin 3, it could be used as a prognosis and diagnosis marker. It is a useful biomarker particularly in the diagnosis of heart failure patients with preserved ejection fraction.

1.5. Biomarkers that define graft rejection

The only treatment option for advanced heart failure patients is heart transplantation (HT). Rejection or graft dysfunction are the major complications accompanied by HT. Endomyocardial biopsy (EMB) post-transplantation is used widely to investigate pathogenic mechanisms of rejection and graft failure.[51] The development of novel molecular biomarkers would help in diagnosing graft rejection at a lower cost for patients. Most patients with organ rejection remain asymptomatic. If symptoms occur, they are similar to heart failure and are not specific. There are three different types of organ rejection - hyper acute, acute cellular, or antibody mediated. Hyper acute normally occurs within hours of heart transplantation and is due to antibodies produced against the organ.[52] The symptoms include myocardial ischemia and graft dysfunction. Acute acellular occurs within 48 h after transplantation and is mainly T cell-mediated and involves inflammatory macrophages and lymphocytes.[53] The use of immunosuppressive agents has reduced the occurrence of acellular rejection. Antibody-mediated rejection occurs within 24 hours of heart transplantation. It is mainly mediated by donor-specific antibodies and tissue injury is promoted by cytokines and macrophages.[54] The accuracy of endocardial biopsy remains low in focal diseases of the myocardium and hence there is an increased requirement for specific and sensitive biomarkers that could detect organ rejection at an earlier stage.

Cardiac troponins are one of the highly specific biomarkers that could trace myocardial damage independent of its etiology. Cardiac troponins are present exclusively in cardiomyocytes. An increase in cardiac troponin levels is seen in acute coronary syndromes and other cardiovascular anomalies.[55] In the surveillance of graft rejection too, cTn could be employed as a potential biomarker. Cardiac troponins levels are elevated immediately following heart transplantation and are often a predictor of allograft survival. Studies measured the levels of hs-cTnT in 141 recipients and found that the mortality rate was higher in patients who had hs-cTnT levels above the median.[56] hs-cTnI could predict acute rejection within 60 days from heart transplantation. hs-cTn shows greater sensitivity than cTn during acellular rejection. Using cardiac troponins, specifically hs-cTn,[57] as a biomarker in the clinical setting of graft surveillance is highly recommended.

Other molecular approaches have been detected to diagnose organ rejection following heart transplantation. Some of them are circulating cell-free DNA (cfDNA), exosomes, and non-coding RNA. cfDNA normally forms during cell death and can detect cellular damage.[58] Normally after transplantation high levels of cfDNA circulate within donor blood. Studies observed that the plasma donor-derived cfDNA, dd-cfDNA levels could predict the possibility of organ rejection.[59] The levels of dd-cfDNA were significantly higher in patients with acellular rejection or antibody-mediated rejection. Also, it is very well correlated with EMB based grade of rejection.[60] dd-cfDNA had exhibited excellent sensitivity and specificity for the diagnosis of rejection starting from 28 days post heart transplantation. More clinical trials are warranted to validate the use of dd-cfDNA as a biomarker during rejection surveillance.

Another biomarker that was studied for surveillance of rejection post-HT is exosomes.[61] Exosomes are small membrane-bound extracellular vesicles that carry proteins, nucleic acids, and other smaller molecules. A study found that patients experiencing acellular rejection or antibody-mediated rejection had exosomes enriched with micro-RNAs like miR-142−3p, miR-92a-3p, and miR-339−3p compared to patients without rejection.[62] Two urinary exosomes were also validated for predicting rejection in kidney transplantation and ensuring high predictive performances.[63] Since exosomes are produced by different cells and under different pathological conditions, using exosomes as noninvasive biomarkers to discriminate patients with rejection will be complicated.

Another potential biomolecule that could be developed as a biomarker and a therapeutic target is the non-coding RNAs. The expression of several micro-RNAs is different in patients with acute cellular rejection.[64] For instance, several micro RNAs were proposed to predict normal and rejecting heart and they had a strong correlation with their tissue expression.[65] Though promising, a large prospective multicenter cohort study is needed to validate the use of micro RNAs as biomarkers.

Other tissue molecular biomarkers under investigation include gene profiling of the fresh, frozen, and formalin embedded tissues and genomic profiling of peripheral blood leukocytes. The plasma proteome could provide an advanced way of predicting a heart rejection.[66] However, these approaches still need to be actively investigated. The presence of additional molecular biomarkers along with the established EMB protocol could help to reduce the late detection of heart transplantation rejection.

Fig. 1 illustrates the biomarkers in use for different purposes in CVDs Fig. 2: Graphical abstract exhibiting the multifaceted role of cardiovascular biomarkers

2. The way forward

The presence and the levels of cardiac biomarkers help clinicians to predict the risk for developing CVDs and provide high risk patients to start lifestyle modifications and medications. This could help prevent the progression of CVDs which would have otherwise left unnoticed and ended in the terminal stage heart failure. As discussed in the review, there are a lot of biomarkers that specifically and sensitively facilitates in predicting the prognosis and diagnosis of CVDs. Given the complexity of CVDs warrants testing the levels of multiple biomarkers to improve the accuracy. A study done to evaluate adverse cardiovascular outcome in type 2 diabetic patients observed the combination of NT-proBNP, osteopontin, and MMP3 had high predictive performances, reinstating the importance of employing multiple biomarkers.[67] Combining novel blood protein biomarkers (ApoBApoA1, KLKB1, LPa, MMP9) with traditional ones (age, sex, cholesterol, blood presure) improved 10-year MI risk assessment.[68]

The significance of the cardiac biomarkers increase in case of individuals getting treated for chronic diseases other than CVDs and who also face the danger of developing CVD complications and dying of sudden heart failure. The screening of biomarkers in such individuals help in managing symptoms under control. The importance of the use of biomarkers has been further realized recently in COVID 19 patients. The biomarkers have largely helped clinicians to stratify these patients based on the risk for developing CVDs and lowering the mortality in COVID 19 patients.

There are many limitations too that defy the specificity and sensitivity of biomarkers. Using metabolites as biomarkers must be cautiously evaluated since the nutrients, nutritional status, and the medication can influence the levels of metabolites irrespective of

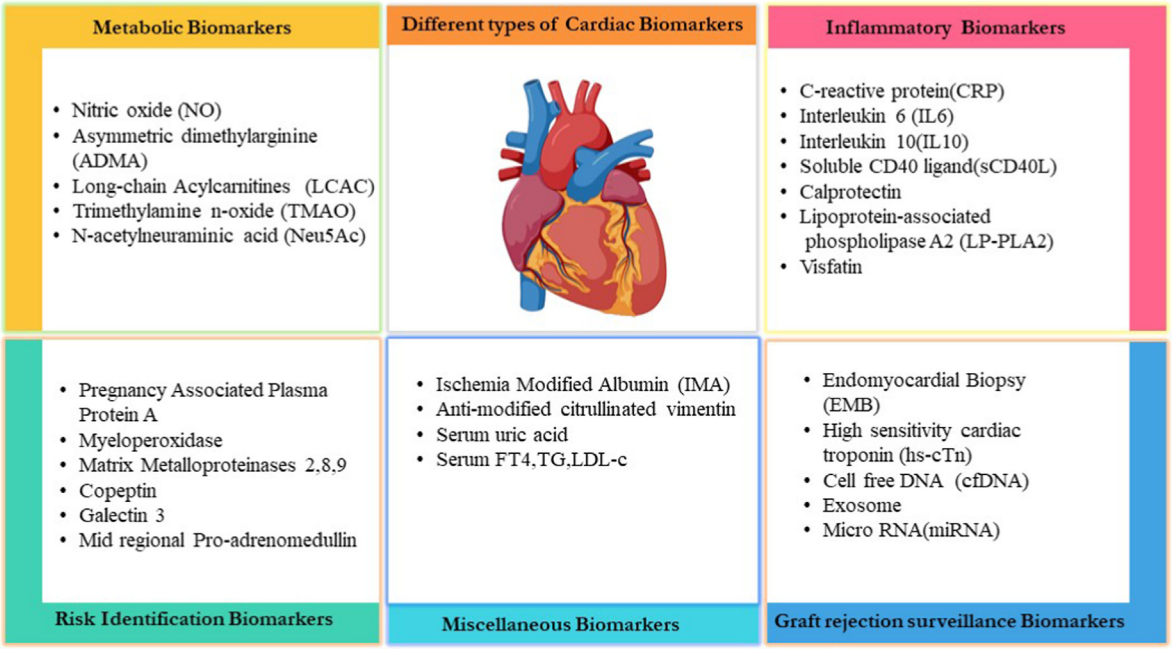

Fig. 1. Illustrates the biomarkers in use for different purposes in CVDs and associated complications.

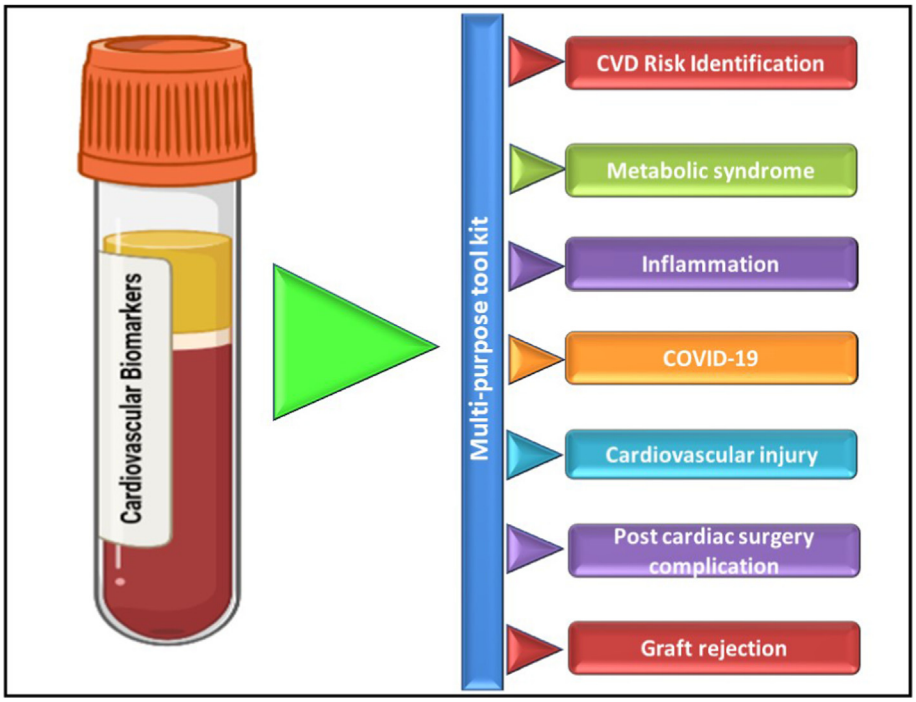

Fig. 2. Graphical abstract exhibiting the multifaceted role of cardiovascular biomarkers.

the disease conditions and hence can vary during the follow-up. Also, whether these biomarkers are the cause, or the consequence of a particular disease condition could not be rightfully evaluated. The newly identified biomarkers like exosomes, miRNAs and nucleotides still require validation using larger cohorts. However, the advantages of cardiac biomarkers are many when compared to the limitations. Clinically evaluating biomarkers in larger cohorts of patients within different populations will help in identifying a gold standard cardiac biomarker.

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