REVIEW

# Incorporating biomarkers into clinical trials in cardiovascular medicine

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**Abstract:** Developing novel therapies and establishing clinical benefits over conventional therapies has become a highly competitive and a costly endeavor. In this regard, biomarkers have become increasingly important in accurately assessing both efficacy and safety of these novel therapies. While the use of biomarkers has proved vital in cardiovascular research and disease management, current established biomarkers are inadequate in capturing and accounting for the entire cardiovascular disease process and responses to therapeutic interventions. Substantial effort has been made in exploring and clinically evaluating novel biomarkers. The present review aims to shed light on some of the novel cardiovascular biomarkers being investigated, including high sensitivity C-reactive protein, proprotein convertase subtilisin/kexin type 9, P-selectin, secretory phospholipase $A_2$, lipoprotein-associated phospholipase $A_2$, and growth differentiation factor 15, and illustrate their relevance to clinical research.

**Keywords:** hs-CRP, PCSK9, P-selectin, sPLA$_2$, Lp-PLA$_2$, GDF-15

## Introduction

Significant strides have been made in cardiovascular medicine over the past 50 years. According to the National Vital Statistics Report, "diseases of heart" is one of only two major causes of death with a steady decline in death rate since 1958 in the United States, with the other being cerebrovascular disease.[1] In contrast, death rates of other major causes of death have been either remained stagnant or increased. And yet, cardiovascular disease continues to be the leading cause of death in the US, accounting for 24.2% of all mortalities. Despite the wide array of highly effective treatments available for cardiovascular diseases, further research is clearly needed to address unmet needs by better understanding the optimal use of current therapies and developing novel therapies.

In clinical research, biomarkers play a crucial role in gathering efficacy and safety data, especially when study duration and sample size is limited. Biomarker is defined by the National Institutes of Health as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[2] Biomarkers may be physical characteristics such as blood pressure, heart rate, and waist circumference, or biochemical characteristics such as serum cholesterol, breath alcohol, and urine albumin. Furthermore, a "valid" biomarker is defined by the Food and Drug Administration pharmacogenomics guidance as:

a biomarker that is measured in an analytical system with well-established performance characteristics and for which there is an established scientific framework or body of evidence that elucidates the physiologic, toxicologic, or clinical significance of the test results.[3]

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In order for a biomarker to be valid, and therefore practical, certain criteria must be met. For one, reference limits must be clearly established so that any abnormality can be clearly captured. In addition, the biomarker must have limited interindividual variability so that the reference limits are applicable across the entire target population. Thirdly, any prognostic or diagnostic ability of a biomarker must have some added value to currently available methods, whether it is improved accuracy, reliability, speed, or cost. Also, changes to the biomarker must have an associated clinical significance such that the change leads to a change in treatment or treatment dose. Establishing a valid biomarker requires intensive research and clinical trials to prove that the above criteria are met. However, biomarkers offer significant benefits not only in clinical practice, but also in clinical trials and are now indispensable tools in clinical research.

In clinical trials, biomarkers may be used for a wide variety of purposes. For example, biomarkers allow investigators to screen for patients that most likely benefit from an investigational therapy. In the development of Herceptin™ ([trastuzumab]; Genentech, Inc., San Francisco, CA, USA), an antibody against human epidermal growth factor receptor 2 (HER2) for the treatment of breast cancers, investigators recruited only patients with HER2-positive breast cancer to maximize the efficacy of the investigational drug.[4] Alternatively, measuring biomarkers at select time points allow investigators to predict long-term clinical outcomes in patients. The Framingham risk assessment is one such biomarker that allows the prediction of the 10-year risk of having a heart attack based on a one-time assessment of biomarkers including age, gender, cholesterol levels, smoking status, and blood pressure.[5] In addition, biomarkers may detect rare safety concerns in clinical trials that would otherwise not present clinically without a large sample size or a long follow-up period. For all clinical trials investigating novel drugs, monitoring the QTc intervals in electrocardiograms has become a standard for identifying risks of fatal cardiac arrhythmias.[6] Drugs that increase the QTc interval raise concerns for fatal ventricular tachycardia as a possible side effect. As illustrated in the examples above, appropriate use of validated biomarkers in clinical trials offers innovative approaches in gathering much needed efficacy and safety data with limited resources and study duration.

The current challenge in the utilization of biomarkers in cardiovascular research, however, is that currently validated biomarkers do not accurately predict clinical outcomes in a significant proportion of patients.[7] Approximately 40% of patients with below-average low-density lipoprotein cholesterol (LDL-C) still die today from cardiovascular diseases, despite LDL-C being widely recognized as a key clinical measure and target of therapy for improving cardiovascular outcomes. Cardiovascular disease is a multifaceted disease that involves disease processes such as atherosclerosis, myocardial disorders, and blood disorders. Therefore, one biomarker should not be expected to reflect the variety of biological processes that are involved in cardiovascular disease. Furthermore, many of the biomarkers used in cardiovascular research are affected by a wide range of factors including age, gender, race, pregnancy status, use of medications, diet, exercise, time of day, and fasting state.[8] With so many compounding factors affecting these biomarkers, the use of these biomarkers for the assessment of treatment efficacy or safety can be highly suspect in clinical settings where not every one of these factors can be controlled. While the use of biomarkers has proved vital in cardiovascular research so far, novel biomarkers are clearly needed to better identify the therapeutic needs and benefits not captured by currently available biomarkers. To address this concern, the present review aims to shed light on some of the novel cardiovascular biomarkers, including high sensitivity C-reactive protein (hs-CRP), proprotein convertase subtilisin/kexin type 9 (PCSK9), P-selectin, secretory phospholipase $A_2$ (sPLA$_2$), lipoprotein-associated phospholipase $A_2$ (Lp-PLA$_2$), and growth differentiation factor 15 (GDF-15), and illustrate their relevance to clinical practice and research.

## hs-CRP

hs-CRP has gained much attention as a potential biomarker for assessing the risk of a recurrence of a cardiovascular event in patients who have experienced myocardial ischemia[9] Although C-reactive protein (CRP) is an acute phase reactant, the chronic levels that are present at low concentrations as measured by hs-CRP are thought to predict clinical outcomes in these patients. CRP has a plasma half-life of 19 hours, and the concentration of hs-CRP can range anywhere from less than 1 mg/L to 3 mg/L or greater. CRP is produced predominantly by hepatocytes in response to cytokines, with interleukin 6 (IL-6) being the most potent of the cytokines. IL-6, on the other hand, is released by leukocytes in response to vascular smooth muscle damage, including atherosclerosis. Therefore, the level of CRP is thought to reflect the amount of damage and atherosclerosis on the vasculature.

The function of CRP in heart disease is very complex and is not yet fully understood. CRP is a mediator of inflammatory pathways and is thought to contribute to the

development of atherosclerosis via macrophage polarization and facilitation of monocyte adhesion and transmigration into the vessel wall.[7] In addition, CRP is thought to bind and recruit LDL-C to the sites of vascular injury. The clinical evidence, on the other hand, for the use of hs-CRP as a predictor for the recurrence of a cardiovascular event is numerous. In the Cholesterol and Recurrent Events (CARE) trial, use of pravastatin in patients post-myocardial infarction (MI) showed significant reduction in the level of CRP and the risk of a cardiovascular event, regardless of the changes in LDL.[9] The study result showed a 75% higher relative risk of recurrent events in patients with CRP levels in the highest quintile compared to those in the lowest quintile (relative risk =1.77, $P$=0.02). In the Multiple Risk Factor Intervention Trial (MRFIT), a randomized prospective trial investigating the effect of a multifactor intervention program in the primary prevention of heart disease in high-risk men, a significant correlation was observed between hs-CRP and cardiovascular mortality over an average follow-up period of 7 years.[10] The correlation was observed not just in men, but also in women in the Women's Health Study (WHS).[11] The study found that, in healthy women, elevated hs-CRP was a strong predictor of cardiovascular events with a relative risk of 2.0, compared to only 1.3 for elevated LDL-C. While the exact molecular interaction by which CRP exerts its effect is yet to be clarified, the clinical evidence does suggest that CRP is a potentially useful biomarker in cardiovascular medicine and research.

Unfortunately, the validity of hs-CRP as a predictive biomarker has not been consistent in clinical trials. In the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial, the study established that the use of statins in patients with low LDL still provide significant mortality benefit among patients with elevated hs-CRP (>2 mg/L).[12] However, the study was criticized for not having a low hs-CRP group, and therefore not being able to determine whether hs-CRP levels contributed to the observed effect of rosuvastatin.[13] Furthermore, post hoc analysis of the study data was not able to detect any correlation between hs-CRP levels and statin response.[14] In the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) trial, no correlation was observed between hs-CRP and response to statin therapy or the risk of future cardiovascular event.[15] The findings of the PROSPER trial were further supported in the Heart Protection Study (HPS) trial, which demonstrated reduction in cardiovascular events with statin use regardless of hs-CRP levels.[16] As a result, monitoring hs-CRP levels has not yet been accepted as part of routine procedures in treating cardiovascular diseases.

The reasons for the inconsistency in the findings across so many clinical trials may be due to the fact that hs-CRP is affected by a multitude of cardiovascular and non-cardiovascular factors.[17] For example, the baseline level of hs-CRP appears to vary across ethnic groups, with the highest levels observed in African Americans, followed by Hispanics, south Asians, whites, and the lowest in east Asians. Furthermore, women tend to have higher baseline hs-CRP compared to men, and hs-CRP increases with age regardless of the presence of cardiovascular disease. Additionally, adipose tissue can be a significant source of IL-6, which stimulates CRP production and affects baseline levels of hs-CRP.[18] In fact, a clinical trial has found that the reliability of hs-CRP as a predictor of cardiovascular events is significantly lower in obese patients.[19] Other factors known to influence hs-CRP levels include age, diabetes, hypertension, oral contraception, exercise, alcohol use, periodontal disease, diet, environmental pollutants, and smoking.[7] Moreover, hs-CRP can have significant day-to-day variations. An analysis of the data from the National Health and Nutrition Examination Survey (NHANES), a survey conducted by the Centers for Disease Control, has found that CRP can change by an average of 46.2% over a 19-day period.[20] Of the patients classified as elevated in the first measurement, 32% of patients were within normal limits after 19 days. To further complicate the matter, 50% of all adults and 41% of 20-year-olds have an elevated hs-CRP level (>2 mg/L).[17] The appropriate use of hs-CRP is still unknown due to the high variability observed across many clinical trials.

Because of the lack of a clear consensus in the findings across clinical trials, the expert panel for the US Preventive Services Task Force recommended against routine testing of hs-CRP for primary prevention of cardiovascular disease in 2002.[21] The panel did recommend, however, testing in intermediate-risk patients with a 10-year risk of 10%–20%. This recommendation is supported by observations in clinical trials where patients with atherosclerosis and a baseline hs-CRP of 3 mg/L had a 50% higher likelihood of experiencing a future cardiovascular event compared to those with a baseline hs-CRP of less than 1 mg/L.[7] This finding highlights the importance of not utilizing hs-CRP as an independent risk factor, but rather as a supplement to other more established cardiovascular biomarkers such as LDL, blood pressure, and others. At the moment, the appropriate use of hs-CRP in clinical trials remains uncertain other than as a biomarker for inflammatory activity. Much research is still needed to

establish its diagnostic or prognostic capabilities as clinical endpoints.

## PCSK9

PCSK9 is an enzyme expressed by the liver, intestines, and the kidneys that plays a role in the degradation of LDL receptors in the liver.[22] Upon binding to the membrane-bound LDL receptor, PCSK9 induces a lysosomal destruction of the LDL receptor, thereby reducing the ability of the liver to uptake and metabolize LDL from circulation. PCSK9 was first discovered through a genetic analysis of patients with familial hypercholesterolemia, which found that a gain of function mutation strongly correlated with hypercholesterolemia. A loss of function mutation correlated with lower incidence of cardiovascular diseases. PCSK9 is involved in the accumulation of triglycerides in visceral adipose tissues as well. Expression of PCSK9 is activated primarily in response to low levels of intracellular cholesterol, but can also be stimulated by other factors such as insulin. Conversely, PCSK9 expression is downregulated in response to inflammation, LDL-C, and fasting. The half-life of PCSK9 is 5 minutes, but may be longer in the absence of LDL receptors. PCSK9 is being investigated not only as a biomarker, but also as a therapeutic target for the treatment of hypercholesterolemia. Inhibition of PCSK9 would allow increased expression of LDL receptors in the liver, thus increasing the metabolism of circulating LDL-C. Since its discovery in 2003, PCSK9 has been studied extensively to establish its clinical relevance and many clinical trials are underway to develop agents against PCSK9.

With no therapeutic agents currently on the market to effectively target PCSK9, the majority of the clinical evidence behind the use of PCSK9 as a biomarker is derived from case studies in patients with mutations of the gene.[23] In the Atherosclerosis Risk in Communities (ARIC) trial, a retrospective trial involving over 12,000 patients, the loss of function mutation of the PCSK9 gene was found to be associated with a 28% reduction of LDL-C and 88% reduction in the risk of heart disease among African Americans, and a 15% and a 47% reduction, respectively, among whites.[24] A similar reduction in LDL-C levels was observed even among children with the mutations in the Bogalusa Heart Study.[25] Interestingly, one of the most popular classes of cardiovascular medications, statins, has been found to upregulate both PCSK9 expression and LDL receptors in a dose- and potency-dependent manner. Administration of atorvastatin 10 mg and 40 mg has been reported to increase plasma PCSK9 levels by 7.4% and 34%, respectively.[26] A more potent statin, rosuvastatin 20 mg, has been reported to increase PCSK9 levels by 28%–35%.[27] The increased expression of PCSK9 in response to statin therapy likely attenuates the therapeutic benefit of statins on LDL-C reduction. Therefore, combining a PCSK9 inhibitor with statin use is expected to have a synergistic effect in lowering LDL through upregulation of LDL receptors without increasing PCSK9 activity. The effects of other lipid lowering agents, such as fibrates and ezetimibe, on PCSK9 expression level has been inconclusive so far.[22] These positive findings have stimulated intense efforts to develop novel inhibitors of PCSK9 as a treatment option for cardiovascular diseases, as evidenced by over 20 clinical trials involving PCSK9 at the time of writing the current review.

## P-selectin

P-selectin is a cell adhesion molecule that mediates the initial recruitment of leucocytes to the inflammatory site.[28] When platelets or endothelial cells are activated by stimuli (eg, inflammatory cytokines, thrombin, histamine or oxidized LDL), P-selectin is translocated to the cell surface. Once migrated to the cell surface, P-selectin binds to P-selectin glycoprotein ligand-1 (PSGL-1) and sialyl-Lewis$^x$, which are constitutively expressed in all leukocytes. The interaction between P-selectin and PSGL-1 serves as the point of initiation of the interaction between platelets, endothelium, and leukocytes. The expression level and degree of glycosylation of PSGL-1 may differ between subtypes of leukocytes, which render endothelial selectivity in recruiting different subtypes of leukocytes to lesions.[29]

P-selectin has two functional forms: membrane-bound P-selectin on platelets and endothelial cells, and soluble P-selectin (sP-selectin) circulating in the blood. sP-selectin is a truncated form of P-selectin, lacking the transmembrane and intracellular domains. The origin of sP-selectin includes platelets and endothelial cells via cleavage of the membrane-bound P-selectin, as well as direct expression.[30] As with membrane-bound P-selectin, sP-selectin has been shown to exert proinflammatory,[31,32] prothrombotic,[33] and procoagulant activities.[34] Elevated levels of sP-selectin are seen in patients with vascular diseases,[31,35] hypercholesterolemia,[36] hypertension,[37] unstable angina,[38] exuberated stroke,[39] and aortic valve stenosis.[40]

P-selectin has been demonstrated to be a key initiator in the development of atherosclerosis via the formation of platelet–monocyte aggregates (PMA).[41–43] P-selectin on the surface of activated platelets binds PSGL-1 on the monocytes to form PMA. PMA binds to the endothelium at a higher affinity than monocytes and delivers cytokines

and procoagulant microparticles (MPs) at the site of inflammation in the vasculature. This results in upregulation of procoagulant tissue factors that may spur fibrin deposition and thrombus formation.[44] As expected, elevated PMA levels are seen in acute coronary syndrome (ACS) patients[45] and increase the risk of cardiac events in non-ST segment elevation MI patients.[46,47]

Another critical role of P-selectin in atherosclerosis is the prerequisite step of leukocyte recruitment through a dynamic adhesion cascade that includes tethering, rolling, firm adhesion, and transmigration of leukocytes into the endothelium.[48–52] P-selectin expressed on stimulated endothelial cells mediates leukocyte tethering and rolling by binding to PSGL-1 present on the leukocyte surface.[53] P-selectin acts as the braking process to decelerate fast-flowing leukocytes in the blood stream. Decelerated leukocytes roll along and interact with the endothelium to further stimulate an inflammatory response, including expression of other adhesion molecules, such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1.[54] Once firm adhesion is achieved through these adhesion molecules, leukocytes infiltrate into the arterial vessel wall to initiate the formation of the atherosclerotic plaque.

P-selectin is also involved in thrombus formation. Interaction between P-selectin and its ligand is essential for thrombus formation in two ways: P-selectin on the activated platelets in the thrombus tethers monocytes, and sP-selectin binds to PSGL-1 on leukocytes to induce the release of MPs.[34] Through P-selectin-PSGL-1 binding, the activated platelets in thrombi recruit and activate monocytes, much like in the case with PMA. Additionally, MPs are recruited to the thrombi by the interaction between P-selectin on the activated platelets in thrombi and PSGL-1 on the MPs, which further stimulates coagulant and inflammatory mechanisms.[55,56] Through tethering of monocytes and generation of MPs, P-selectin mediates thrombus formation and ultimately contributes to arterial occlusion and ischemia.

Increased expression of P-selectin is observed in a variety of cardiovascular diseases, including peripheral artery diseases, ACS, and stroke. Elevated levels of P-selectin have been observed on the endothelial cells overlying atherosclerotic plaques[57] and on circulating platelets in patients with peripheral arterial diseases.[58,59] Patients with unstable angina, congestive heart failure, or acute MI show significantly elevated levels of sP-selectin and platelet-bound P-selectin as compared to healthy subjects.[60] In addition, patients with unstable angina exhibit significantly higher levels of P-selectin expression in coronary atherectomy specimens

than patients with stable angina.[61,62] Elevated levels of sP-selectin were also observed in patients with vascular disorders such as hypertension and hypercholesterolemia.[36,37] In patients with acute cerebral infarction, P-selectin and sP-selectin levels have been shown to be elevated compared to healthy individuals, and to correlate with an increase in phospholipase $A_2$ (PLA).[63,64] Taken together, P-selectin appears to have a correlation not only with onset of disease, but also with progression of disease and shows great promise as a cardiovascular biomarker.

Because of the association between escalating levels of sP-selectin and pathological states, sP-selectin is being investigated as a diagnostic biomarker for cardiovascular diseases.[65,66] For instance, plasma sP-selectin levels may be an indicator for successful thrombolysis in acute MI.[66] Acute MI elevates P-selectin levels by approximately fourfold, but diminishes within 24 hours after successful thrombolytic therapy using tissue plasminogen factor. In contrast, sP-selectin levels remain high in patients with unsuccessful thrombolysis. Similarly, cell surface P-selectin and plasma sP-selectin have been suggested as potential markers of platelet activation in a number of disease models, such as ischemic heart disease, rheumatic mitral stenosis, and atrial fibrillation.[67]

P-selectin may not only predict disease outcome and progress, but also cardiovascular complications following therapeutic interventions. Plasma sP-selectin levels are elevated in patients with myocardial ischemia, and further rise after reperfusion therapy by nearly 30% over a 1-week period post-reperfusion.[68] This increase leads to PLA formation, and is believed to significantly contribute to post-angioplasty restenosis. Furthermore, P-selectin levels rise post-revascularization in the heart, which is thought to reflect the extent of reperfusion injury caused by the therapeutic intervention.[69,70] In support of this notion, inhibition of P-selectin via administration of recombinant soluble P-selectin glycoprotein ligand-Ig in a swine model of reperfusion post-myocardial-ischemia was able to maintain myocardial blood flow at a pre-ischemia level for 2 hours post reperfusion.[70] In contrast, only 65% of the myocardial blood flow was maintained in the control group ($P<0.05$). In addition, P-selectin inhibitors have demonstrated a significant increase in endothelium-dependent vasorelaxation of coronary arteries, reduction in infarct size, and reduction in the impairment of ejection fraction in various animal models of MI.[70–72] In addition to playing a complimentary role in assessing cardiovascular disease state, P-selectin also may have a niche role in monitoring complications post-intervention,

including impeding atherosclerosis development, preventing thrombus growth and fibrin deposition,[42,73–75] alleviating myocardial reperfusion injury,[43,76] and reducing restenosis.[77]

Intriguingly, a number of currently available therapies are known to have effects on P-selectin, and may explain some of the therapeutic benefits observed with these agents beyond their intended effect. In a clinical trial involving 148 patients, patients taking 100 mg aspirin showed significantly lower plasma sP-selectin levels post-percutaneous coronary intervention (PCI) after a loading dose of 600 mg clopidogrel, compared to those not on aspirin ($P$=0.004).[78] The presence of the CYP2C19*2 allele, which is responsible for clopidogrel metabolism, had no impact on the inflammatory markers in these patients, suggesting clopidogrel did not influence sP-selectin levels. Heparin and its analogues are also known to inhibit P-selectin activity.[79] In an in vitro experiment using blood of healthy volunteers, heparin at therapeutic plasma concentrations suppressed P-selectin expression on the surfaces of thrombin-stimulated platelets by nearly 80% ($P$<0.001), and enoxaparin did so by approximately 60% ($P$=0.0014).[79] The inhibitory action is thought to be mediated through inhibition of P-selectin binding to sialyl-Lewis[x].[80] Attempts have been made to produce a heparin analogue without the anticoagulant effect for P-selectin inhibition.[81] Statins are another class of therapeutic agents known to inhibit P-selectin activity. In a crossover trial involving 32 patients with atherosclerotic ischemic stroke, platelet P-selectin level was significantly lower after 12 weeks of treatment with simvastatin 20 mg, compared to the levels after a 12-week washout period.[82] The reduction in P-selectin expression was independent of changes in LDL. The author proposed that the decrease in P-selectin level might be attributable to a statin-mediated anti-inflammatory mechanism, as evidenced by reduced CRP levels. In another clinical trial involving coronary artery disease (CAD) patients, patients receiving statins for 6 months had approximately 30% lower plasma sP-selectin levels compared to those not on statins ($P$<0.0001).[83] The sP-selectin reduction was accompanied by over 50% reduction in hs-CRP levels ($P$<0.001). Although the exact mechanism behind statin-induced inhibition of P-selectin is yet to be elucidated, the findings above suggest targeted inhibition of P-selectin, in combination with current standard of care, may prove to be a novel and effective treatment for cardiovascular diseases.

## PLA

Two members of the PLA enzyme family, sPLA$_2$ and Lp-PLA$_2$, have been evaluated as biomarkers of cardiovascular risk in population-based studies inclusive of apparently healthy individuals and patients with established coronary heart disease (CHD) such as ACS and MI.[84] PLAs are capable of generating various lipid mediators by modification of LDL-C, and are thought to mediate vascular inflammation and atherosclerosis. Lp-PLA$_2$ and sPLA$_2$ are molecularly and biochemically distinct and thus their roles and underlying mechanisms of action in the blood and within the vascular wall are likely to be different.[85,86] The prognostic values of circulating levels of Lp-PLA$_2$ and sPLA$_2$ have been intensively investigated and they have been suggested as independent predictors of cardiovascular events in various patient populations, which will be further discussed below.

## Lp-PLA$_2$

Although still controversial, Lp-PLA$_2$ is thought to contribute to the progression of atherosclerosis. Lp-PLA$_2$ is predominantly bound to LDL in humans.[87,88] A recent study on hypercholesterolemic minipigs showed that elevated Lp-PLA$_2$ activity increased levels of lysophosphatidylcholine, oxidized LDL, and activity of proinflammatory genes in coronary plaque macrophages, which hasten the progression of atherosclerosis.[89]

The suggestion of Lp-PLA$_2$ as a predictor for cardiovascular diseases originated from the West of Scotland Coronary Prevention Study (WOSCOPS) in which each of the 580 men presenting with a coronary event (ie, nonfatal MI, death from CHD, or a revascularization procedure) were matched for age and smoking status with two control subjects (1,160 controls in total) from the same cohort without a coronary event.[90] Lp-PLA$_2$, CRP, and fibrinogen levels were measured at baseline along with other traditional risk factors. The findings in this study showed a positive correlation between increased circulating Lp-PLA$_2$ levels and the risk of coronary events in hypercholesterolemic men even after accounting for age, systolic blood pressure, and lipoprotein levels. On the other hand, other traditional risk factors had weaker correlations after accounting for the aforementioned variables. The prognostic value of Lp-PLA$_2$ for cardiovascular events has also been demonstrated in patients with stable CAD.[91–93] In addition to predicting the risk of a cardiac event, Lp-PLA$_2$ has been suggested as a risk predictor for restenosis. In a recent study involving 326 patients eligible for stent placement, elevated baseline levels of Lp-PLA$_2$ (>200 ng/mL) were associated with an increased risk for restenosis after 1 year.[94] Lp-PLA$_2$ has been studied as a biomarker for a variety of cardiovascular diseases and may prove to be useful in both clinical practice and research.

One caveat, however, is that Lp-PLA$_2$ has not been shown to be a reliable cardiovascular biomarker for patients with ACS. The data from the North Wuerttemberg and Berlin Infarction Study-II (NOBIS-II) study suggested that Lp-PLA$_2$ levels might predict cardiovascular complications only in a subgroup of ACS patient with moderately increased N-terminal pro-brain natriuretic peptide (NT-proBNP).[95] Another study in patients with MI reported a positive association between elevated levels of Lp-PLA$_2$ and 1-year cardiac mortality.[96] However, subsequent large studies did not show such consistent findings as the aforementioned studies. A meta-analysis of Lp-PLA$_2$ levels in 1,362 ACS patients from the FRagmin and Fast Revascularization During Instability in Coronary Artery Disease (FRISC II) study, 904 ACS patients from the Global Use of Strategies to Open Occluded Arteries IV (GUSTO IV) study, and 435 apparently healthy controls showed no association between Lp-PLA$_2$ levels and recurrent MI or mortality.[97] Similarly, analysis of Lp-PLA$_2$ levels from 3,648 ACS patients at admission and from 3,265 patients at 30 days after ACS in the PRavastatin Or atorVastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction (PROVE IT-TIMI22) trial found no association with cardiovascular events.[98] However, the patients with Lp-PLA$_2$ activity in the highest quintile at the 30-day follow-up did present increased risk of recurrent cardiovascular events compared to patients with Lp-PLA$_2$ activity in the lowest quintile. Further adding questions to the prognostic value of Lp-PLA$_2$, the recent Phase III STabilisation of Atherosclerotic plaque By Initiation of darapLadib TherapY (STABILITY) trial concluded that darapladib, a specific Lp-PLA$_2$ inhibitor, failed to meet its efficacy endpoints in patients with stable CHD as defined by having at least one of the following: previous MI, previous PCI, previous coronary artery bypass graft, or multivessel CHD.[99] In the 15,000-patient trial, darapladib failed to meet the primary endpoint of reduction in major adverse cardiovascular events (comprised of cardiovascular death, MI and stroke, or all-cause mortality).[99] In the Stabilization of Plaques using Darapladib – Thrombolysis in MI 52 (SOLID-TIMI52) trial, darapladib also failed to meet the primary endpoint of a reduction of major coronary events in patients with ACS.[100] As such, Lp-PLA$_2$ activity has not been conclusive in its ability to predict clinical outcomes in patients with ACS.

Much work is still needed to fully understand Lp-PLA$_2$ and its association with cardiovascular disease. For example, some of the studies mentioned above compare Lp-PLA$_2$ activity level rather than the mass of circulating Lp-PLA$_2$, but not much is understood regarding which method of measurement more reliably predicts outcome. Although the prognostic value of Lp-PLA$_2$ for cardiovascular risk has not been consistent among all populations, the currently available data suggest that measurement of Lp-PLA$_2$ could improve cardiovascular risk assessment beyond traditional risk factors in diseased patients.

## sPLA$_2$

In contrast to Lp-PLA$_2$, the mechanism for sPLA$_2$ in promoting atherosclerosis is more solid and conclusive.[101] Circulating levels of sPLA$_2$ increase greatly during systemic inflammatory conditions. The hydrolytic action of sPLA$_2$ generates various lipid mediators from the lipid deposits in the plaques on the arterial wall. The lipid mediators further trigger local inflammatory cellular response, which leads to atherosclerotic disease progression. In addition, sPLA$_2$ may potentiate the binding and retention of LDL on the plaques by increasing the affinity of apolipoprotein B100 on LDL particles for glycoaminoglycans and proteoglycans on the arterial wall. Furthermore, sPLA$_2$ is implicated in the production of isoprostanes, which exhibit strong mitogenic activity and induce platelet aggregation and vasoconstriction. Moreover, a study using C57BL/6 mice given a high-fat diet demonstrated that overexpression of sPLA$_2$ leads to increased susceptibility to atherosclerosis.[101] These findings led to a host of clinical trials to elucidate the clinical significance of sPLA$_2$ as a cardiovascular biomarker.

The prognostic value of sPLA$_2$ has been addressed in a number of studies on apparently healthy subjects,[102,103] patients with stable CAD,[98,104–106] ACS patients,[107,108] patients with unstable angina,[109] and patients with acute MI.[110] The European Prospective Investigation of Cancer (EPIC)-Norfolk Study, which was a two-nested case-control study, found that increased sPLA$_2$ levels and activity correlated with the rate of first occurrence of a coronary event during 6 years of follow-up in relatively healthy individuals.[102,103] In another study involving 142 patients with angiographically proven stable CAD, the presence of stable CAD was associated with higher sPLA$_2$ levels compared to control individuals.[104] In the study by Koenig et al, increased sPLA$_2$ levels after PCI were associated with subsequent CAD events.[108] The utility of measuring sPLA$_2$ activity was further supported by another large cohort in the PEACE study.[111] The study, involving 3,708 patients with stable CAD, found that increased sPLA$_2$ activity was associated with increased risk of adverse events such as cardiovascular death, MI, heart failure, and stroke during an average of 4.8 years of follow-up.[111] The usefulness

of sPLA$_2$ as a predictive biomarker has also been investigated in patients with ACS. A trial involving 1,032 ACS patients found a significant correlation between sPLA$_2$ mass and activity (r=0.67), and an association between both measurements and the risk of cardiovascular events.[105] Similar findings have also been reported in the French Registry of Acute ST Elevation or Non–ST-Elevation Myocardial Infarction (FAST-MI) trial.[110] The plasma sPLA$_2$ level has also been shown to be an independent predictor of clinical coronary events in patients with unstable angina.[109] In a 255-patient study, sPLA$_2$ levels were significantly higher in patients with unstable angina than in those with stable angina or healthy subjects, and had a significant correlation with a 2-year risk of developing clinical coronary events.[109] Furthermore, the investigators of the 446-patient study called Global Registry of Acute Coronary Events (GRACE) reported that sPLA$_2$ activity was associated with the risk of death and MI in a stepwise fashion with increasing tertiles of sPLA$_2$.[107]

Currently, no commercially available assay can quantify the activity of the individual sPLA$_2$ isoenzymes, which limits the use of sPLA$_2$ in clinical practice. However, with a clear understanding of the molecular mechanism for its role in cardiovascular disease and a strong correlation with clinical outcomes in a variety of cardiovascular diseases, sPLA$_2$ presents as a potentially highly useful endpoint biomarker in clinical research.

## GDF-15

GDF-15 is a member of the transforming growth factor superfamily. GDF-15 is present in macrophages and many types of cardiovascular cells, and its expression is triggered by a variety of inflammatory mediators, such as oxidative stress, oxidized LDL, and various cytokines.[112,113] Cardiomyocytes can also produce GDF-15 in response to these triggers, as well as mechanical stretch.[112,114,115] Similarly, adipocytes are known to release GDF-15 in response to oxidative stress.[116,117]

While not much is known about its function, the pathophysiologic role of GDF-15 in cardiovascular disease is thought to be a part of the defensive mechanism by myocytes in response to ischemia or increased myocyte stress. Rapid elevation of GDF-15 in the ischemic area after coronary artery ligation was observed in murine models of ischemia and reperfusion injury lasting several days.[112,118] Likewise, increased levels of GDF-15 are observed in patients with atherosclerosis.[119] Furthermore, infusion of recombinant GDF-15 into infarcted myocardium suppresses the inflammatory response, suggesting a role as a counter-regulatory cytokine.[120]

Increased levels of GDF-15 have been associated with increased overall and cardiovascular mortality in patients with ST segment elevation MI,[121,122] non-ST segment elevation MI,[123] and stable CHF.[124] In three large clinical trials involving non-ST elevation-ACS patients (the GUSTO-IV, FRISC II, and Invasive versus Conservative Treatment in Unstable coronary Syndromes [ICTUS] sub-studies), higher GDF-15 level was independently associated with higher mortality rate over a 2-year follow-up period in the first two studies, and a 5-year follow-up period in the other.[123,125,126] Furthermore, the FRISC II study found an association between elevated GDF-15 and increased rate of MI recurrence in patients with CAD (n=230), as well as the severity of coronary disease on angiogram.

While the role of GDF-15 in cardiovascular disease is clear, the utility of GDF-15 as a cardiovascular biomarker has not been so straightforward. In contrast to the GUSTO-IV, FRISC II, and ICTUS trials, the AtheroGene study found that patients with stable angina (n=1,352) and ACS (n=877) with GDF-15 greater than 1,800 ng/L are not at increased risk of nonfatal MI compared to those with low GDF-15.[127] These patients were, however, at an increased risk of CHD mortality. The findings suggest that circulating GDF-15 levels are a stronger risk marker for mortality than for nonfatal coronary events in high-risk populations. In support of this notion, a 646-patient study called the Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) study also found GDF-15 to be a better predictor of mortality than of nonfatal CV events.[128] In another study involving patients with acute MI, GDF-15 was inferior to high-sensitivity troponin T and brain natriuretic peptide in detecting acute MI.[128,129] However, GDF-15 was superior to both markers in predicting all-cause mortality. Furthermore, GDF-15 was as reliable as high-sensitivity troponin T in predicting death or future acute MI in patients presenting with chest pain without acute MI. The findings above raise questions as to whether GDF-15 can be a diagnostic tool for cardiovascular diseases. Even so, all evidence points to GDF-15 being a valuable biomarker in the context of cardiovascular medicine and warrants further research regarding its application in clinical practice and research.

## Conclusion

The field of cardiovascular medicine has become a progressively competitive and costly field for the development of novel therapies and establishing clinical benefits over currently available therapies. Despite the challenges, cardiovascular disease is the leading health concern in the United States

and warrants continued research and development for novel treatment options to address this still unmet need. To do so, biomarkers have become increasingly important in accurately assessing both the efficacy and safety of these novel therapies. Current established biomarkers, such as LDL-C and blood pressure, fail to fully capture and account for the entire cardiovascular disease process and to provide clear targets for lowering mortality. An undeniable need exists for novel biomarkers to fully understand the disease process and identify new treatment targets for the improvement of clinical outcomes in patients with cardiovascular disease. Significant efforts are underway to explore such novel biomarkers, including hs-CRP, PCSK9, Lp-PLA$_2$, sPLA$_2$, and GDF-15. Whether the aforementioned biomarkers prove useful is still up to debate and more clinical trials are necessary to establish their validity. Nonetheless, new biomarkers are sure to play a critical role in both clinical practice and research, and a deep understanding of the benefits and limitations of these biomarkers will be crucial for clinicians and researchers alike.

## Disclosure

The authors report no conflicts of interest in this work.

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