journal homepage: https://jablm.acclmp.com/

Journal of Applied Biochemistry & Laboratory Medicine (2022) 03 (1):15-21

Review article

The evolution of cardiac biomarker assays in supplantation of complicated multimodal diagnostic approach towards cardiac disease.

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Keywords:
Cardiac biomarkers, AMI, Troponins, NT-ProBNP, Micro RNA, Proteomics.

How to cite this article
Mukherjee S. The evolution of cardiac biomarker assays in supplantation of complicated multimodal diagnostic approach towards cardiac disease. Journal of Applied Biochemistry & Laboratory Medicine 2022; 03 (1):15-21

Access link for this article
https://jablm.acclmp.com/2022/03/01_15-21.pdf

ABSTRACT

With evolution cardiac biomarkers has become an integral part of diagnosis and management of cardiovascular disease. Among the many markers myocardial enzymes were discovered first; gradually they were replaced by several myocardial proteins and peptides like troponins, ischemia modified albumin, natriuretic peptides etc. Cardiac troponins not only detect the disease at earliest with highest degree of sensitivity and specificity but also these tests are easily available even at bed side. So rightfully troponins have become the cardinal diagnostic parameter of 4th universal definition of acute myocardial infarction. The latest addition to the cardiac markers family are proteomics and micro RNA. Micro RNA are very stable in the circulation and can easily predict cardiovascular events in diseased and apparently healthy individuals as well. But their invention also comes with a challenge of implementation in clinics in a timely and cost-effective manner.

INTRODUCTION

In the field of modern diagnostic research based clinical mecine, biomarkers have become an essential tool for development of diagnostic and therapeutic approach. Biomarkers are developed for objective measurement of some parameters which can indicate a certain biological state under normal physiological or abnormal pathological state both. [1] Furthermore, when used with certain objective direction they can indicate the disease pathology upto genetic and molecular level. disease. Accordingly, evolution of cardiac biomarkers have enabled to define the acute myocardial infarction (AMI) in a new way by the American College of Cardiology and the European society of Cardiology .[2] In brief the important features of an ideal cardiac marker can be enumerated as follows:

- Both high sensitivity and specificity for indication of the AMI.
- Minimally affected by normal or pathological functioning of other organs.
- Can be measured easily and uniformly in different conditions.
- Rise and fall with ischemia should be well associated in a concerted manner.
- Have an optimal turnaround time i.e less than 60 min. [3]

In general, cardiac markers can be broadly classified according to the pathogenesis of acute coronary syndrome and prognosis (vide figure-1).

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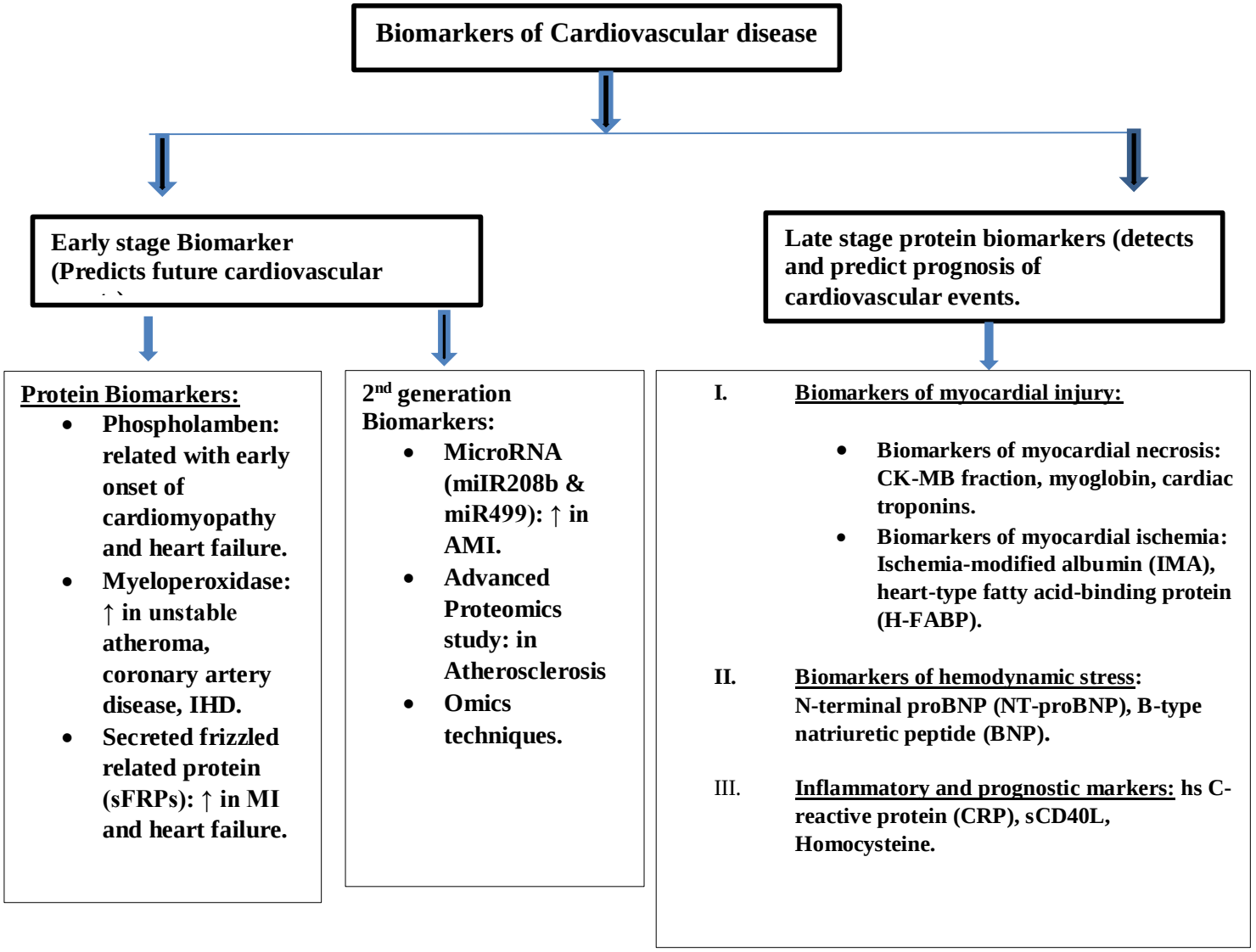

Figure-1: Flowchart showing significance of biomarkers at various levels in the pathogenesis of cardio vascular events. [3, 4]

Evolution of cardiac biomarkers:

Since 1950 the journey for developing biomarkers of cardiovascular disorders, particularly that for myocardial infarction (MI), has been initiated and thence it has been continuously developed by defining a battery of biomarkers e.g aspartate amino transferase (AST) in 1954, lactate dehydrogenase (LDH) in 1955 and creatine kinase (CK) in 1960 at the initial stages. However, these enzyme assays had their certain limitations and so their replacement was taking place using other smaller molecules which could be detected much earlier as an indication for the beginning of actual myocardial necrosis (vide Table-1). The developments proceeded with separation of the isoforms of CK by electrophoresis in 1972 that made the CK-MB fraction an extremely useful tool for an early detection of AMI. At present, this has been further supplemented with CK-MB mass /index, Myoglobin, Troponin I and T for making diagnosis of AMI at the earliest possible time. [4] (Vide figure-2). The following discussion explains these in the context of their uses and limitations.

Biomarkers of Myocardial Necrosis:

Myoglobin— This is a single polypeptide chain heme containing protein which generally stores and transports oxygen in muscular tissues. Myoglobin is abundantly found in cardia tissues and is released as early as 1 hr within any injury to myocardial tissues. It release is maximum at 8 to 10 hours after injury and its level is normalized after 24 hours of the injury. Due to early rise and peak myoglobin has been reported as a sensitive early indicator for AMI, albeit its availability in other muscular tissues limits its specificity for the cardiac tissues significantly. However, this limitation can be overcome to some extent by making a serial measurement every 1 to 2 hours. Non cardiac conditions where myoglobin is increased are vigorous exercise, Rhabdomyolysis, progressive muscular dystrophies, shock, renal failure and cardiac conditions where myoglobin does not increase are cardiac catheterization and congestive cardiac failure without AMI. Serial sampling every 1-2 hours can increase the sensitivity and specificity. If there is no rise seen in serum myoglobin levels in two samples analyzed 2 to 4 hours apart, it virtually rules out AMI. [5]

Marker	Time of rise	Time of peak	Time to achieve baseline	Remarks
CK (MB)	4-6 h	24 h	72 h	Useful in diagnosis of early infarct, re-infarct
AST	2-4 h	48 h	4 days	Low specificity
LDH (1& 2)	12 h	72 h	7-12 days	Low specificity
Troponin T	2-6 h	24 h	10-14 days	High cardiac specificity
Troponin I	2-6 h	24 h	7 days	Complete cardiac specificity

Table -1: Markers of acute myocardial infarction.

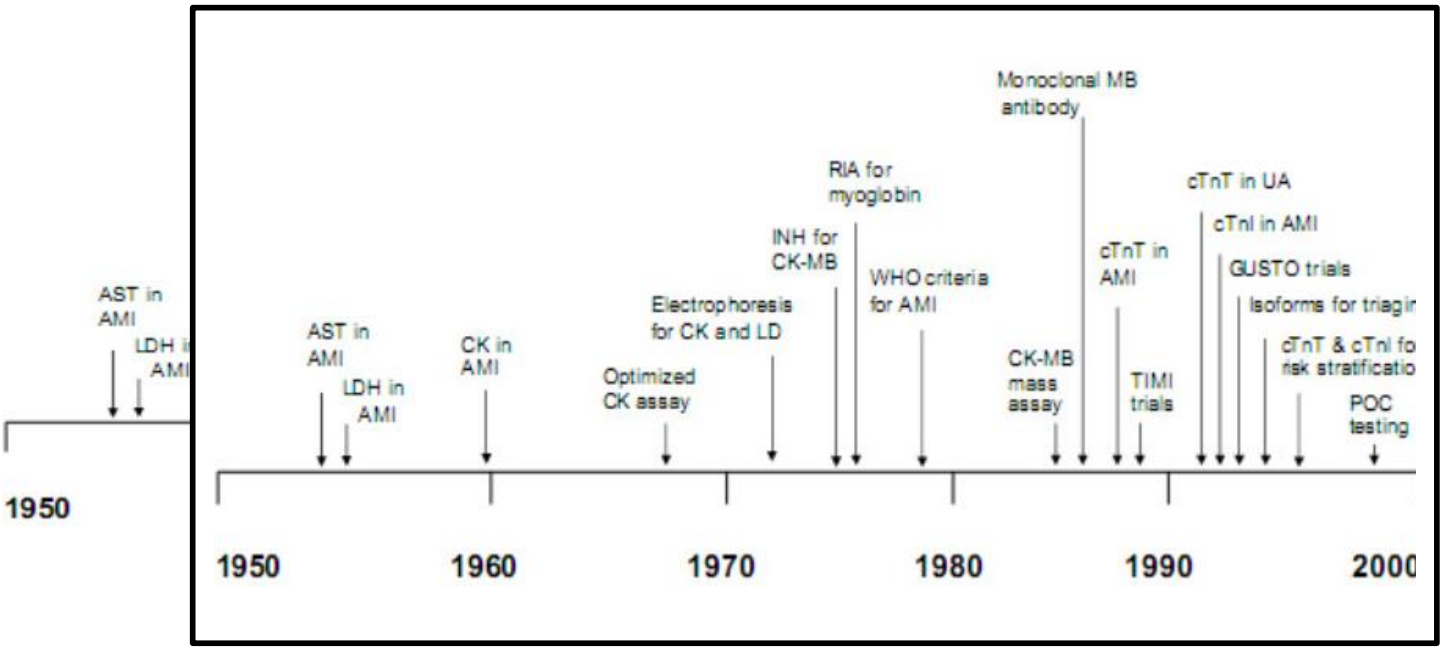

Figure-2: Evolution of cardiac biomarkers. [3]

Creatine Kinase and Creatine Kinase-MB:

Electrophoretic and other immunological techniques have enabled the separation of the creatine kinase enzyme into its three iso-enzyme forms namely CK-MM, CK- MB, and CK-BB. As soon as this separation occurred, the CK-MB fraction soon replaced the measurement of total CK activity as the former is more specific for the cardiac tissues and can be measured both by its mass and enzyme activity. Total mass of CK – MB is of much importance as it orms nearly 30% of total CK in the myocardium. CK-MB starts appearing in the bloodstream 4 to 6 hours after onset of cardiac injury and it peak is generally found between 10 and 12 hours after the start if the incident. This isozyme held its cardinal position as an early detector of MI for many years. Using this isoenzmy as the biomarker clinicians could detect cases of AMI most optimally between 6 and 48 hours after which it gets cleared from the circulation. This also poses its limitation as cases arriving later thatn 48 hours of their MI cannot be diagnosed by using this enzyme. Moreover, a serial measurement procedure generally gives more accurate information of the duration and extent of AMI than a single measurement.[6]

The relative index is a very useful index to differentiate between CK from the myocardium, skeletal muscle, or from neural damage.

It is calculated as:

Relative index = CK−MB/Total CK × 100

A relative index of > 2.5 to 3 indicates very likely heart damage, lower index suggesting skeletal muscle damage. [7]

CK mass assay is found to be of better diagnostic value for the detection of re-infarction after an index event than the CK, CK-MB enzyme activity and relative index. Mass assay circumvents many problems like of false-high results owning to contribution by CK-BB & atypical forms of CK [macro-CK]. [8]

Cardiac Troponins:

Cardiac Troponins (cTn) which control the calcium-mediated interaction of actin and myosin have been found to be expressed in three isoforms in cardiac tissues i.e troponin C, troponin I and troponin T. Among these three, the troponin C (18 kD) has no cardiac specificity as it has been reported to exists in all muscle tissues where it acts mainly as calcium binding protein. Troponin T (39kD) is predominantly of cardiac origin where it performs the act of anchoring troponin complex to the tropomyosin strand. Unlike troponin C, Troponin T (26.5kD) is completely cardiac specific.

If compared with CK-MB, both troponin-I and troponin-T have highly degrees of specificity and sensitivity for indicating the early cardiac tissue damage and hence are more useful for detection of infarction. So, with the evolution of the cardiac biomarkers, these two have been selected as preferred biomarker for the diagnosis of AMI. The initiation period and progress of AMI is confirmed with a rise and/or fall in cTn values with the major criteria of having at least one value above the 99th percentile URL. Furthermore, it is more indicative if it is coupled with a high clinical and/or ECG likelihood of myocardial ischemia (Class I recommendation from the ACC/AHA task force on diagnosis of AMI). [9] However, it should be kept in mind that a wide range of cardiac causes leads to elevation of cardiac troponin values > 99th percentile URL. (vide figure-3) which should be kept in mind for differential diagnosis of AMI using these diagnostic parameters.

The course of rise and fall of cardiac troponin I follows a typical pattern like all other cardiac biomarkers. It starts increasing in circulation in 2 to 6 hours, reaches its peak at 12 hours, and returns to basal levels in 3 to 10 days. In contrast, troponin-T stays elevated for 12 to 48 hours and falls to normal in 10 Days. [10]

Point of care testing: Troponin T is the most abundant cardiac troponin in the circulation; now widely used in point-of-care testing device considerably at the bedside to exclude cardiac damage in patients with chest pain. But the POCT has its own limitations like Sampling is not standardized, lower Precision, narrow analytical range, and limited utility in patients with low values.

High-sensitivity troponin (hs-Trop) assay: hs troponin assay has shown its marked potentiality for early diagnosis of AMI in patients with non–ST-elevation myocardial infarction (NSTEMI), and allows diagnosis by a single blood test. Some studies have concluded that a single hsTnT level ≤ 6 ng/L indicated a very low risk of AMI, whereas serial levels exceeding 19 ng/L identified patients with risk of adverse cardiac events. [10,11] High value at 0 hour with large increase after 1 hour "rule in" ACS whereas Low value at 0 hour with no change after 1 hour "rule out" ACS. [12]

The hsTn assays worth values also in patients with acute heart failure (AHF) as seen in a study by Xue et al. [13] The patients with hsTnI > 23.25 ng/L during discharge show increased risk of readmission and mortality. Thus troponins also play a significant role in prognosis and management of cardiac dysfunction.

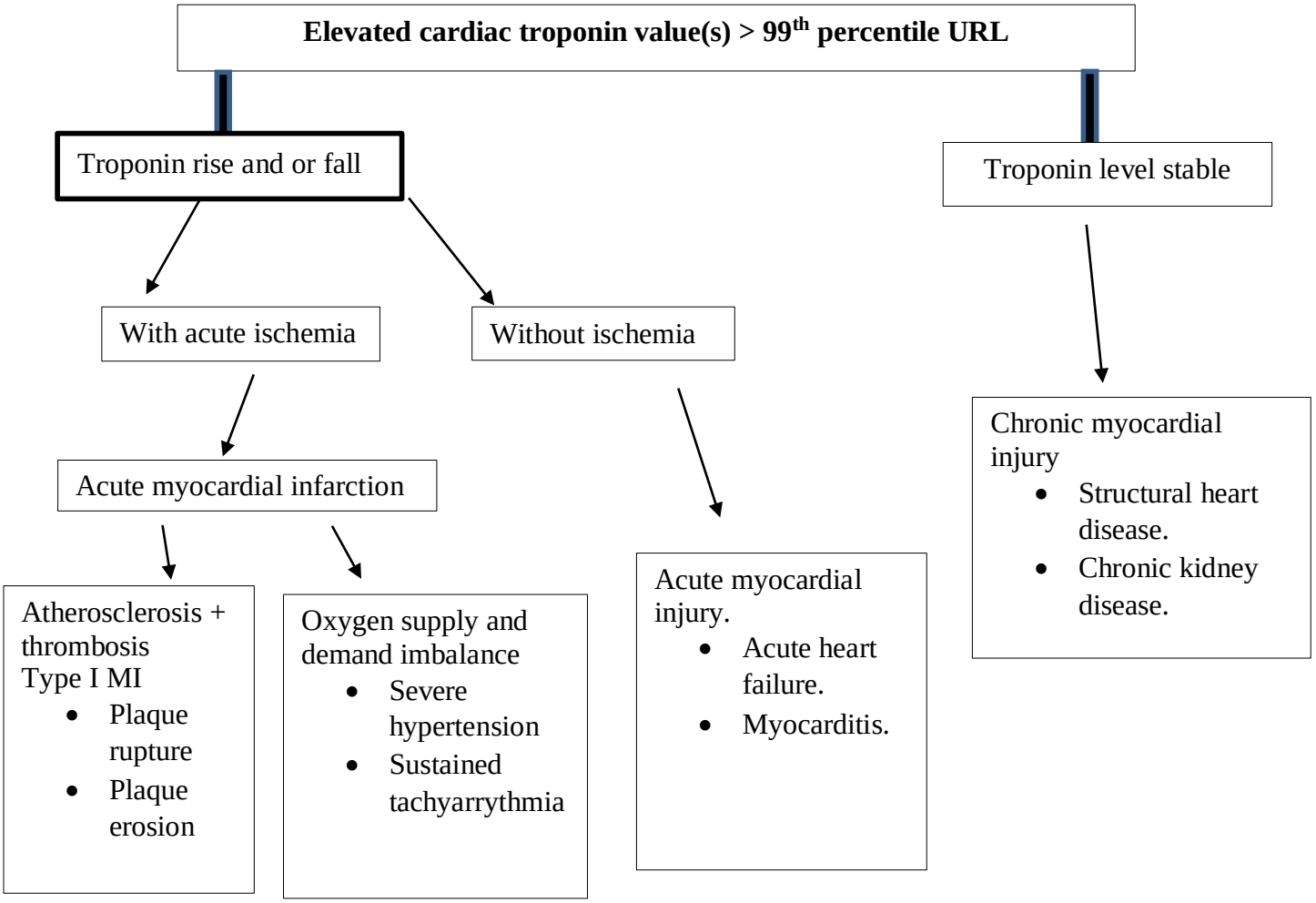

Figure-3: Causes of cardiac troponin elevation in response to myocardial injury.

Markers of Myocardial Ischemia:

Ischemia-Modified Albumin (IMA): IMA is generated in detectable amount when the circulating albumin is exposed to ischemic tissues yielding ischemia modified albumin that has lesser degree of metal binding affinity at its ischemia modified N terminal residues. As all biomarkers, IMA has its own course of rise and fall. It starts increasing within 6 hours of an ischemic attack and is elevated for 12 hours. However, albumin is almost ubiquitous all over the body, IMA is elevated by non-cardiac conditions also such as hypoxia, acidosis, etc of heart as well as some other tissues. [14]

Heart-type Fatty Acid-Binding Protein (H-FABP)—It is a very stable low-molecular-weight (14–
15 kDa) protein present in the myocardial cytoplasm. These are involved in the transport of fatty acids from the cell membrane to the mitochondria for oxidation. Having a smaller size compared to other biomarkers it can easily diffuse through the interstitial space and appear in the circulation as early as 1 to 3 hours of onset of the ischemia induced damage. This biomarker reaches its peak levels within 6 hours, and then returns to normal in 24 hours. Use of H-FABP may improve diagnostic ability, but the presence of easily available specific markers such as troponins has made it an ancillary test only. [15]

Biomarkers of Hemodynamic Stress:

N terminal Pro BNP and B type natriuretic peptide (BNP): Natriuretic peptides are a group of structurally similar molecules having significant differences in their genetic makeups. These low molecular weight proteins, better known as peptides, regulate the balance of sodium and water in the body by modifying their excretion under different physiological and pathological conditions. One of their major functions is the regulation of blood pressure in response to cardiac fluid overload. Both N terminal Pro BNP and B type natriuretic peptide (BNP) are the diagnostic markers for Heart failure. They can predict and rule out heart failure; help in risk stratification, indicate severity; correlates with NYHA Classification of HF and guide management. More to these they are also useful in prediction of post-surgery cardiovascular complications. [16]

NT proBNP (108 a.a.) is precursor to B type natriuretic peptide (32 a.a.). The increased blood flow into the ventricles/pressure creates a stretch in the ventricular wall, which is an inducer of transcription for natriuretic peptides. BNP ≥ 100 pg/mL and Nt-proBNP ≥ 300 pg/mL rule in acute heart failure where as BNP < 100 pg/mL and Nt-proBNP <300 pg/mL rule out AHF. NT ProBNP is superior to BNP in diagnostic efficacy as it has Longer half-life [120 min v/s 20 min], greater in-vitro-stability [48 hours v/s 4 hours]; having higher assay range [0-35,000 pg/mL v/s 0-5000 pg/mL], age specific cut off value (< 50 years = ≥ 450 pg/mL; 50–75 years = ≥ 900 pg/mL; >75 years = ≥ 1,800 pg/mL) and surely it is not affected by neprilysin inhibitors. [17]

Inflammatory and Prognostic Markers:

High sensitive C-reactive protein (hs-CRP): An increased hs-CRP level has been suggested an independent cardiac infraction risk predictor. In hospital indoor patients hs-CRP has been reported to predict an incident of AMI independent of the GRACE risk score. Whereas a value< 1 mg/L shows low risk, values 1 to 3 mg/L and > 3 mg/L indicates a moderate and increased risk respectively 5]. Furthermore, hs-CRP has been established as a simple marker of the magnitude of the inflammatory response to myocardial ischemia in its early phase [18] .

Homocysteine- This is an intermediary amino acid in the amino acid metabolic pathway. This amino acid is particularly liken with atherosclerosis and has been established as an independent risk factor for the development of atherosclerosis. Hyper-homocysteinemia leads to thickening of the intimal tissues that leads to disruption of the elastic lamina. Along with this damage, smooth muscle hypertrophy and platelet aggregation potentiate the vascular injury in a holistic manner. Hence, homocysteine is a useful marker for risk assessment for atherosclerotic tissue damage. Normal homocysteine level in blood is 5–15micromol/L. An increase of 5 micromol/L of homocysteine in serum elevates the risk of coronary artery disease. [19]

Soluble CD40 ligand (sCD40L)—It is a signaling protein that reflects both inflammation and platelet interaction and found to be increased in acute coronary syndrome (ACS). However due to several limitations, it is merely of prognostic value rather than diagnosis. [20]

Early Stage Biomarkers of Cardiovascular Disease:

Invention of early stage biomarkers is crucial in predicting cardiovascular events in future irrespective of the person's physiological condition. Protein profiling using proteomic techniques and micro RNA screening are undoubtedly two important milestones in the evolution path. In addition, data information about the RNA sequences enable an early detection of any abnormal gene expression and thence a dysregulated protein synthesis or protein misfolding provide early cues in early stages of disease.

Development of early stage cardiac markers:

Phospholamban: Arg->Cys mutation at residue 9 in the phospholamban gene (PLN-R9C). has been found to be linked to lethal dilated cardiomyopathy and heart failure (Ceholski et al, 2012). This protein usually functions by inhibiting sarco/endoplasmic reticulum calcium transport ATPase (SERCA) in its dephosphorylated form and thereby regulating the cardiac contractility by. [21]

Myeloperoxidase (MPO): MPO and metalloproteases are proteolytic enzymes that lyse the collagen protein network in the matrix of an atherosclerotic plaque, thereby leading to erosion and rupture of the plaque. This enables these proteolytic enzymes as early biomarker of CVD due to their strong association with atheroma instability. Several studies have already indicated elevated MPO levels can be used as early indicators of coronary artery disease even before detection by angiography or cardiac troponin levels. [22]

Secreted frizzled related proteins (sFRPs): High serum sFRPs level is usually considered as early biomarker for MI, heart failure, and its adverse outcomes. It has a prognostic role in the disease management as well. sFRPs actually serve as Wnt antagonists; so when elevated it activates a pro-apoptotic pathway which leads to AMI. [23, 24]

2nd generation Biomarkers:

Micro RNA: Micro RNAs are evolving as novel biomarkers in the areas of CVD. These are of short length and do not take part in active translation of proteins. Rather they inhibit the gene expression by binding to sites in the untranslated regions of targeted messenger RNAs. Stability and their resistance to changes in pH, temperature, freeze-thaw cycles, along with a long-term storage capability make them important tools for investigation. Furthermore, due to their high tissue specificity, and conservation of their sequences in different species provide them more robustness for their use of early biomarkers. Several methods can be used to measure their levels. Till now, at least four groups of miRNAs are identified which are involved in regulation of the CV system and of them miR-208b, miR499 are very specific for AMI. [25]

"Omics" study: Current research is directed towards invention of new ideal biomarkers for CVDs. Omics study is such an emerging system of biomarkers which allow samples to be analyzed as a global set of macromolecules of DNA, RNA, proteins, lipids, and metabolites that are expressed in different organ systems, such as the cardiovascular system and analysing the normal physiological function as well as pathological events in a holistic manner that are unique in characteristics for each

individual. Their information can be obtained by using plasma, urine, whole blood, and tissues and analysing them holistically using the recent tools of bioinformatics to get maximum information about a particular physiological or pathological state. However, validation of these biomarkers using different methods is essential before translating these biomarkers from the lab into the clinics. [26]

Advancement in proteomics in relation to CVD: This is an extension of the Omics technique where a certain protein believed to play a role in the development or progression of a disease e.g atheroma can be detected more holistically and effectively. Using these technique, all proteins linked to development of atheroma can be studied along with the pivotal and accessory role of each one. Recent techniques like matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry (MS) enabled to use it in more effective way. For example using the studies on proteomics, an overexpression of Haptoglobin and serum amyloid-A have been found to be associated with atherothrombotic ischemic stroke, [27]

Advantages of cardiac biomarkers over multimodal diagnostic approach:

Cardiovascular diseases pose a huge burden over society. They are one of the leading cause of disease associated morbidity and mortality all over the world. So prompt diagnosis and treatment is a must do for CVD management.

For an early diagnosis and management of cardiovascular event a multimodal diagnostic approach is usually needed including the recently developed cardiac biomarker assays, ECG changes, echocardiography and if needed, angiography. As this whole approach is time consuming, it imposes unwanted delay in the initiation of proper treatment during the golden hours of an cardiovascular event. The multidimensional approach also needs movement of the patient from one lab to another that may pose significant trouble for the patient at the acute state of illness. Thirdly and most importantly ECG changes may remain non conclusive and the high end imaging procedures are not available everywhere that may pose severe threat on the patients particularly during very early stage which is considered to be golden hours for the management. All these inconveniences of multimodal diagnostic approach point towards the need of a single test which is easy to avail even at bed site, can accurately diagnose the event at very early stage so that treatment can be started at its golden hours and adverse outcomes can be reversed easily. A single gold standard test definitely reduces the economic burden as well.

Joint Task Force of the European Society of Cardiology, American College of Cardiology Foundation, the American Heart Association, and the World Health Federation (ESC/ACCF/AHA/WHF) redefines the AMI.

Keeping all these factors in mind the Fourth universal definition of myocardial infarction has been modified (2018):

"Detection of a rise and/or fall of cardiac biomarker values [preferably cardiac troponin (cTn)] with at least one value above the 99th percentile upper reference limit (URL) should be considered as **Cardinal criterion** that should be accompanied by one or more ancillary test":

1. New lower bundle brunch block
2. Presence of pathological Q waves in ECG
3. Any imaging evidence of recent loss of the viable myocardium
4. Newly developed regional wall motion abnormality
5. Intracoronary thrombus detection by angiography or autopsy
6. Symptoms of ischemia
7. New or presumed new significant ST-segment–T wave (ST–T) changes. [28]

According to the recent definition cardiac troponin assay is the gold standard test that single handedly "rule in" or "rule out" AMI so does the NT-ProBNP in heart failure. [12, 17] Troponin I not only secures the highest sensitivity and specificity but also it detects the disease at earliest (~2 hours) with utmost accuracy and also available as "point of care test." So one single cardiac biomarker assay can replace the multimodal approach indisputably and save the golden hours of disease management.

Lastly we always say that "prevention is better than cure" and it fits for CVD management also. In CVDs, many of the biomarkers used clinically are late stage biomarkers, indicating the presence of a disease that has already developed. Identifying early stage biomarkers of CVDs is of great importance in preventing these diseases from progression and their associated complications. These include changes at the level of proteins, genes, and miRNAs. Proteomics is a promising tool for the discovery of new biomarkers. The new proteomic tools provide many advantages; first, they require a small volume of sample; secondly these methods use the presence of appropriate and specific antibodies for targeted biomarkers. The use of miRNA expression profiling have also given rise to the discovery of many miRNAs that could be used as biomarkers for CVD.

Conclusion:

Nowadays Cardiac markers have been widely implicated in the diagnosis and risk stratification of patients with chest pain and suspected ACS. Among the markers cardiac troponins have become the marker of choice for patients with ACS. In fact, cardiac troponin has become central to the 4th universal definition of AMI. Now current focus lies on finding appropriate upstream markers which will add a new dimension to the diagnostic approach and a variety of events involved in the process of pathophysiology of acute coronary syndrome so ACS can be predicted even in apparently healthy individual and risk stratification can be done accurately. For this early stage novel cardiac biomarkers like microRNA, Proteomics are evolving nowadays but the challenge lies in implementing their use in the clinic in a timely and cost-effective manner.

Acknowledgement: Nil.

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