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REVIEW ARTICLE

Cardiac Markers as Diagnostic Biochemical Markers in Heart Failure

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ABSTRACT

We are in new era of identifying and treating patients with diseases. Heart disease is the leading cause of mortality in developed countries said as lethal diseases all over the world. In this review, we summarize recent literature focusing on circulating biomarkers that can aid the diagnosis of acute heart failure, facilitate prognostication, and guide disease management. Putative heart failure biomarkers can be broadly and empirically classified into indicators of neuro-hormonal activation (brain natriuretic peptide [BNP] and norepinephrine), markers of myocyte injury and extracellular matrix remodeling, and inflammatory mediators. Other biomarkers at early stages of investigation are also discussed briefly. This review does not cover genomic and echocardiographic biomarkers of heart failure but gives the diagnostic, monitoring and risk of stratification properties of existing and emerging markers of Cardiovascular diseases (CVD's).Cardiac markers are used to predict the increased risk of heart diseases. Among cardiac markers for CVD's risk that have received attention in this review are Troponin, Myoglobin, Creatine kinase, C-Reactive protein and to highlight the clinical usefulness of serial measurement of these markers in heart diseases. The existing cardiac markers and their potentials gives the researchers an insight for new research and to study the emerging markers like Matrix Metaloproteins (MMP), Myeloperoxidase (MPO), homocysteine etc. It is critically important to place Cardiac markers in the temporal context of clinical symptoms and signs. This is a substantial advantage for point of care (POC) testing where availability of biochemical marker is in time frame particularly in (ED) emergency department.

KEYWORDS

Biomarkers, Heart failure, Troponin, Creatine kinase, Point of care testing.

INTRODUCTION

The adverse morbidity and mortality of heart failure patients has been described in both randomized trials and population-based studies[2,3]. The recognition of the dismal prognosis of heart failure has led to greater efforts to identify the condition early and to optimize risk stratification strategies to guide management.

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Epidemiologic studies have identified a number of clinical risk factors for heart failure, including elevated blood pressure, diabetes, renal insufficiency, and coronary heart disease[3-6]. The prediction of heart failure and its associated outcomes can be a challenging task, however. Biomarkers may have potential utility in further improving the diagnostic and prognostic capabilities that clinicians apply in routine practice. The scientific literature on bio-markers for the diagnosis of heart failure and for informing prognosis has grown steadily, triggered by insights into the pathophysiology of

cardiac dysfunction and a greater understanding of the contributing molecular mechanisms. As with any other diagnostic test or prognostic index, the gold standard that any eligible biomarker must satisfy is that it must provide incremental information beyond that offered by simple clinical assessment. Such an expectation must be met before widespread clinical use of a bio-marker can be recommended[6].

Cardiac Markers: "Cardiac markers are substances released from heart muscle when it is damaged as a result of myocardial infarction." Cardiac marker tests identify blood chemicals associated with myocardial infarction (MI), commonly known as a heart attack. The myocardium is the middle layer of the heart wall composed of heart muscle. Infarction is tissue death caused by an interruption in the blood supply to an area. "A biomarker is a substance used as an indicator of a biologic state. It is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." "Biomarker" is a very broad term that refers to parameter reflecting or characterizing a certain biological process. It may include variety of indices/parameters derived from clinical images, physiological tests, tissue biopsies, and even genetic variants, but most often, this term is reserved for blood or urine based assessments, it leak out of injured myocardial cells through their damaged cell membranes into the bloodstream.

History: Total CK designed as a fast, reproducible spectrophotometric assay in the late 1960's.CK isoenzyme are subsequently described as MM, MB and BB fractions.1970's: MB fraction noted to be elevated in and highly specific for acute MI[1] .CKMB now measured via a highly sensitive monoclonal antibody assay. It was felt for a time that quantitative CKMB determination could be used to enzymatically measure the size of an infarct. This has been complicated by release of additional enzymes during reperfusion. As CK-MB assays become more sensitive, researchers come to the paradoxical realization that it too is not totally cardiac specific. The MB fraction is determined to be expressed in skeletal muscle, particularly during the process of muscle regeneration. Myosin light chains were originally isolated and then subsequently abandoned because of specificity issues. Troponin I first described as a biomarker specific for AMI in 1987[1]; Troponin T in 1989. Now the biochemical "gold standard" for the diagnosis of acute myocardial infarction via consensus of ESC/ACC. This work encourages development of other clinical assays for diagnosis and prognosis of a wide spectrum of cardiac diseases Notable examples: BNP (FDA approved in November 2000 for diagnosis of CHF) C-reactive protein[7].

Need: Cardiac biomarker tests are ordered to help detect the presence of ACS and cardiac ischemia and to evaluate their severity as soon as possible so that appropriate therapy can be initiated. It is important to distinguish heart attacks from angina, heart failure, or other conditions that may have similar signs and symptoms because the treatments and monitoring requirements are different. For heart attacks, prompt medical intervention is crucial to minimize heart damage and future complications. Cardiac biomarker tests must be available to the doctor 24 hours a day, 7 days a week with a rapid turn-around-time. Some of the tests may be performed at the point of care (POC) – in the Emergency Room or at the person's bedside. Serial testing of one or more cardiac biomarkers is necessary to ensure that a rise in blood levels is not missed and to estimate the severity of a heart attack. Only a few cardiac biomarker tests are routinely used by physicians. The current biomarker test of choice for detecting heart damage is Troponin. The existing markers for myocardial necrosis, such as cardiac Troponin, Creatine kinase-MB, and Myoglobin are thought to be released into blood following irreversible myocardial necrosis. Thus results of these tests are usually negative for patients with acute coronary syndromes (ACS) who present to the emergency department (ED) within the first 3 hours after the onset of chest pain. Given the need to make early therapeutic

and triage decisions, biomarkers that can be used to diagnose and/or risk stratify ACS patients during their initial ED presentation will be important. Active research in this area has identified several classes of biomarkers that show promise for early detection of disease. These include tests for the presence of acute inflammation and infiltration (e.g., high sensitivity-C-reactive protein, Myeloperoxidase), plaque instability (e.g., pregnancy-associated plasma protein-A, placental growth factor), platelet activation (e.g., whole blood choline, platelet density, CD40 ligand), and myocardial ischemia (e.g., ischemia modified albumin, free fatty acids, serum choline, and B-type Natriuretic peptide)[7].

Characteristics of Ideal Cardiac Markers

Size: smaller markers are released faster from injure tissues. Cellular localization: soluble cytoplasmic marker. Absolute cardiac marker specificity: marker should not exist in other tissues under physiological or pathological conditions. High tissue sensitivity: abundance in cardiac tissue and absence from plasma. Release: its release from myocardium should be complete following the injury and in direct proportion to the size of injury. Stability: marker should reach peak levels shortly after the injury and persists in circulation for few hours to allow a suitable diagnostic window. Clearance: marker should be cleared rapidly to allow diagnosis of recurrent injury. Applications: should permit monitoring of reperfusion, reocclusion and both early and late diagnosis of cardiac injury. Detectability: rapid whole body assay that is quantitative and cost effective must be available for the marker. Sensitivity (also called recall rate in some fields) measures the proportion of actual positives which are correctly identified as such (e.g. the percentage of sick people who are correctly identified as having the condition). Sensitivity = number of true positives / number of true positives + number of false negatives Specificity measures the proportion of negatives which are correctly identified (e.g. the percentage of healthy people who are correctly identified as not having the condition). Specificity = number of true negatives / number of true negatives + number of false positives (True positive: Sick people correctly diagnosed as sick, False positive: Healthy people incorrectly identified as sick, True negative: Healthy people correctly identified as healthy, False negative: Sick people incorrectly identified as healthy)[7].

Types of Biochemical Markers

Prognostic Biochemical markers

Neurohormones

Norepinephrine

A complex series of neuro-hormonal changes take place in response to a low cardiac output and small arterial volume that are characteristic of HF due to systolic left ventricular (LV) dysfunction. Sympathetic nervous activity is increased, myocardial noradrenalin stores are depleted and the $beta_1$ - adrenoreceptors are down regulated and desensitized. An initial increase in adrenergic activity might help maintain the cardiac performance in the short term, with several negative consequences, including increased myocardial contractility, tachycardia, and arterial vasoconstriction, which increases cardiac afterload. The increase in concentrations of circulating norepinephrine is due to its increased release from adrenergic nerve endings and its spillover into the plasma, combined with a reduced uptake by the nerve endings, while only a small amount of circulating norepinephrine originates from the heart[8]. In the Vasodilator-Heart Failure II Trial, which examined several potential predictors of outcome in nearly 750 patients, including baseline LV ejection fraction (EF), peak oxygen consumption during exercise, and cardiothoracic ratio, log plasma norepinephrine remained the only independent predictor of mortality[9]. The Cooperative North Scandinavian Enalapril Survival Study found a positive correlation between mortality and plasma norepinephrine in the group of patients assigned to placebo[10]. In the Studies of Left Ventricular Dysfunction, the median plasma norepinephrine concentration was significantly higher in patients with LV dysfunction than in normal controls[11]. While these historical observations are robust, the need

for bed rest before blood sampling, and for high-performance liquid chromatography, a time-consuming procedure that is not readily available, has limited the clinical use of plasma norepinephrine concentrations.

Renin, Angiotensin, Aldosterone

The activity of the circulatory renin—angiotensin— aldosterone system is central to the maintenance of water and electrolyte balance and blood volume, and the local renin—angiotensin system plays an important role in the pathogenesis of chronic HF[8] . Renin is mainly released by the juxta-glomerular cells in response to renal hypoperfusion and sympathetic activation. Angiotensinogen is cleaved by renin to form angiotensin I, which is converted by the angiotensin-converting enzyme (ACE) into angiotensin II, a stimulator of aldosterone production by the adrenal cortex. However, in patients suffering from chronic HF, these compensatory mechanisms ultimately increase the pre and afterload. While patients with mild chronic HF may have little or no increase in plasma renin or plasma aldosterone concentrations, their activation does predict outcome, although perhaps not as accurately as plasma norepinephrine[9,10].

Natriuretic Peptide

In contrast to other neurohormones that are elevated in chronic HF, ANP and BNP seem to play adaptive counter-regulatory roles. Both hormones are synthesized as amino acid precursor proteins. Pro-ANP is sequestered in atrial storage granules, and cleaved into N-terminal proANP and active hormone ANP upon its release into the circulation. BNP is regulated during gene expression, and is released from ventricular myocytes as N-terminal proBNP (NT-proBNP) and BNP, the active hormone. ANP and BNP, which seem to produce identical effects, relax vascular smooth muscle, dilate arteries and veins, lower blood pressure and ventricular preload, and inhibit sympathetic activity and the renin—angiotensin—aldosterone system. They also increase glomerular filtration and inhibit sodium reabsorption by the kidney, promoting natriuresis and diure-sis. BNP is more reliable than ANP or N-terminal proANP in the evaluation of chronic HF and plasma concentrations of BNP are currently used as diagnostic and prognostic markers in patients with chronic HF[12]. It is particularly noteworthy that, by multiple variable analysis, BNP was a stronger predictor of mortality than NHYA functional class, ANP, norepinephrine, LVEF, or age[13]. In Val-HeFT, norepinephrine, BNP, aldosterone, plasma renin activity (PRA), big endothelin (ET)-1, and ET-1 were assayed at baseline in 4300 patients. By multiple variable analysis, BNP was most closely correlated with mortality, followed by norepinephrine and PRA[14]. BNP is also a predictor of survival in patients with acutely decompensated HF. In the Acute Decompensated Heart Failure Registry, the relation between BNP concentration on admission to the hospital and in-hospital mortality was linear[15].

Tissue Markers

Markers of Collagen Deposition

Procollagen type III aminoterminal peptide Processes involved in cardiac remodeling other than myocyte injury, such as the turnover of interstitium, fibroblasts, and collagen, play an important role[16]. Norepinephrine and angiotensin II stimulate the production of collagen *in vitro*. The prognostic value of the serum concentrations of procollagen type III amino-terminal peptide was confirmed in a sample of over 260 patients with chronic HF enrolled in the Randomized Aldactone Evaluation Study[17].

Markers of Myocyte Injury

Troponin is attached to the protein tropomyosin and lies within the groove between actin filaments in muscle tissue. In a relaxed muscle, tropomyosin blocks the attachment site for the myosin crossbridge, thus preventing contraction. When the muscle cell is stimulated to contract by an action potential, calcium channels open in the sarcoplasmic membrane and release calcium into the sarcoplasm. Some of this calcium attaches to troponin which causes it to change

shape, exposing binding sites for myosin (active sites) on the actin filaments. Myosin binding to actin forms cross bridges and contraction (cross bridge cycling) of the muscle begins.

Physiology: Both cardiac and skeletal muscles are controlled by changes in the intracellular calcium concentration. When calcium raises, the muscles contract, and when calcium falls, the muscles relax. Troponin is a component of thin filaments (along with actin and tropomyosin), and is the protein to which calcium binds to accomplish this regulation. Troponin has three subunits, TnC, TnI, and TnT. When calcium is bound to specific sites on TnC, tropomyosin rolls out of the way of the actin filament active sites, so that myosin (a molecular motor organized in muscle thick filaments) can attach to the thin filament and produce force and/or movement. In the absence of calcium, tropomyosin interferes with this action of myosin, and therefore muscles remain relaxed[18]. Troponin I have also been shown to inhibit angiogenesis in vivo and in vitro. Individual subunits serve different functions: Troponin C binds to calcium ions to produce a conformational change in TnI Troponin T binds to tropomyosin, interlocking them to form a troponin-tropomyosin complex Troponin I binds to actin in thin myofilaments to hold the troponin-tropomyosin complex in place. Troponin C (red) binds Ca2+, which stabilizes the activated state, where troponin I (yellow) is no longer bound to actin. Troponin T (blue) anchors the complex on tropomyosin. Troponin is found in both skeletal muscle and cardiac muscle, but the specific versions of troponin differ between types of muscle. The main difference is that the TnC subunit of troponin in skeletal muscle has four calcium ion binding sites, whereas in cardiac muscle there are only three. The actual amount of calcium that binds to troponin varies from expert to expert and source to source.

Release of Troponin

When a cardiac myocyte dies, CK-MB passes rapidly from the cytoplasm into the circulation and is cleared. In contrast, most of the Troponin within the myocyte is found in the structural elements of the cell, so when necrosis occurs there is a steady leaching of Troponin into the circulation. Consequently, Troponin remains in the circulation for several days after a cardiac event.

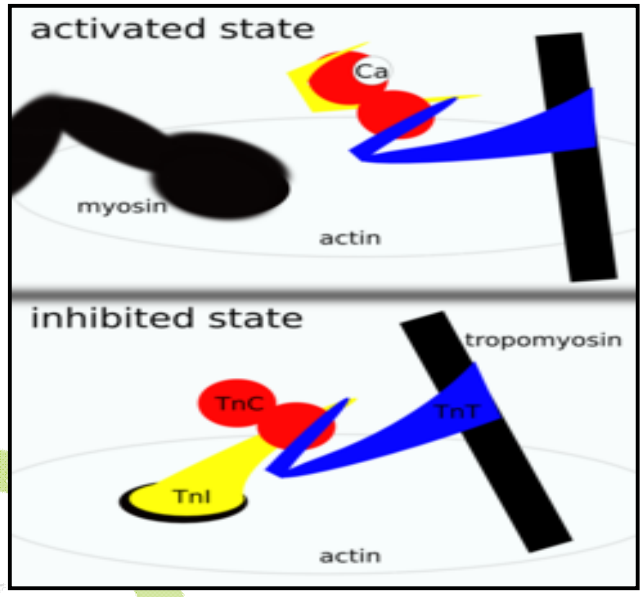

Figure 1: Troponin

Despite extended searching, there is currently no evidence that the cardiac troponins may be produced by tissues other than myocardium. However, the presence of cardiac troponin, while indicating that cardiac injury has occurred, provides no information as to the mechanism of injury. Cardiac troponin concentrations may rise in conditions unrelated to ischaemic damage such as pericarditis, trauma and sepsis. Such rises provide no information about the likelihood of future ischaemic cardiac disease. When associated with coronary artery ischemia even low concentrations of cardiac troponin predict an adverse outcome. This is regardless of whether the other WHO criteria for the formal diagnosis of myocardial infarction are met. The pathophysiological mechanism for these acute coronary syndromes is the presence of an unstable coronary plaque, with release of micro-emboli causing focal myocardial necrosis with release of cardiac troponin. The increased mortality is a reflection of a large thrombus separating from the unstable plaque. This

improved understanding of the mechanism of the acute coronary syndrome, has led to a proposal to redefine myocardial infarction, using the presence of a cardiac biochemical marker, with some evidence of coronary artery ischaemia, as the central diagnostic criterion. Release of cardiac Troponins in acute myocardial infarction. The zone of necrosing myocardium is shown at the top of the figure, followed in the middle portion of the figure by a diagram of a cardiomyocyte that is in the process of releasing biomarkers. Most troponin exists as a tripartite complex of C, I, and T components that are bound to actin filaments, although a small amount of Troponin is free in the cytoplasm. After disruption of the sarcolemmal membrane of the cardiomyocyte, the cytoplasmic pool of troponin is released first (left-most arrow in the bottom portion of figure), followed by a more protracted release from the disintegrating myofilaments that may continue for several days (three-headed arrow). Cardiac troponin levels rise to about 20 to 50 times the upper reference limit (the 99th percentile of values in a reference control group) in patients who have a "classic" acute myocardial infarction (MI) and sustain sufficient myocardial necrosis to result in abnormally elevated levels of the MB fraction of creatine kinase (CK-MB). Clinicians can now diagnose episodes of microinfarction by sensitive assays that detect cardiac troponin elevations above the upper reference limit, even though CK-MB levels may still be in the normal reference range (not shown)[18].

The troponins are a family of proteins found in skeletal and heart muscle (cardiac) fibers. There are three different types: troponin C (TnC), troponin T (TnT), and troponin I (TnI). Together, these three proteins regulate muscular contraction. Cardiac-specific troponins I and T (cTnI and cTnT) are troponins that are found only in the heart. They are normally present in very small to undetectable quantities in the blood. When there is damage to heart muscle cells, cardiac-specific troponins I and T are released into circulation. The more damage there is, the greater their concentration in the blood. The troponin test measures the amount of cardiac-specific troponin I or T in the blood and is used to help determine if an individual has suffered a heart attack. When a person has a heart attack, levels of cardiac-specific troponins I and T can become elevated in the blood within 3 or 4 hours after injury and may remain elevated for 10 to 14 days.

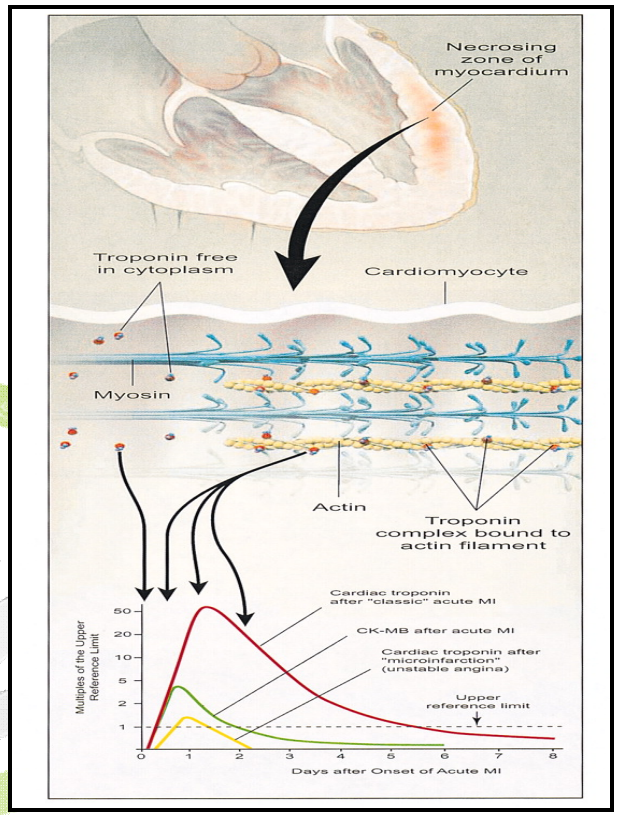

Figure 2: Release of Troponin

No test preparation is needed. Troponin tests are primarily ordered to evaluate people who have chest pain to see if they have had a heart attack or other damage to their heart. Either a cardiac-specific troponin I or troponin T test can be performed; usually a laboratory will offer one test or the other. Troponin tests are sometimes ordered along with other cardiac biomarkers, such as CK–MB or myoglobin. However, troponins are the preferred tests for a suspected heart attack because they are more specific for heart injury than other tests (which may become positive in skeletal muscle injury) and remain elevated for a longer period of time. The troponin test is used to help diagnose a heart attack, to detect and evaluate mild to severe

heart injury, and to distinguish chest pain that may be due to other causes. In those who experience heart-related chest pain, discomfort, or other symptoms and do not seek medical attention for a day or more, the troponin test will still be positive if the symptoms are due to heart damage. A cardiac-specific Troponin I or T test will usually be ordered when a person with a suspected heart attack first comes into the emergency room, followed by a series of Troponin tests performed over several hours. It is sometimes ordered along with other tests such as CK, CK–MB, or Myoglobin. Because troponin is specific to the heart, even slight elevations may indicate some degree of damage to the heart. When a person has significantly elevated troponin levels and, in particular, a rise and/or fall in the results from a series of tests done over several hours, then it is likely that the person has had a heart attack or some other form of damage to the heart. When someone with chest pain and/or known stable angina has normal troponin values in a series of measurements over several hours, then it is unlikely that their heart has been injured. Troponin values can remain high for one to two weeks after a heart attack. The test is not affected by damage to other muscles, so injections, accidents, and drugs that can damage muscle do not affect cardiac troponin levels. Troponin may rise following strenuous exercise, although in the absence of signs and symptoms of heart disease, it is usually of no medical significance.

Currently Used Biomarkers

C-Reactive Protein Crp stands for C-Reactive Protein. It's a protein produced by our immune system in response to supposed antigens or invaders in our body. CRP shows inflammation occurring in our arteries. Hardening arteries are associated with atherosclerosis which is a major risk factors for coronary heart disease.

B-Type Natriuretic Peptide (BNP), now known as B-type natriuretic peptide (also BNP) or GC-B, is a 32 amino acid polypeptide secreted by the ventricles of the heart in response to excessive stretching of heart muscle cells (cardiomyocytes). BNP is named as such because it was originally identified in extracts of porcine brain, although in humans it is produced mainly in the cardiac ventricles. BNP is co-secreted along with a 76 amino acid N-terminal fragment (NT-proBNP) which is biologically inactive. BNP binds to and activates the atrial natriuretic factor receptors NPRA, and to a lesser extent NPRB, in a fashion similar to atrial natriuretic peptide (ANP) but with 10-fold lower affinity. The biological half-life of BNP, however, is twice as long as that of ANP, and that of NT-proBNP is even longer, making these peptides better targets than ANP for diagnostic blood testing.

Lactate Dehydrogenase: It catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+. It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply, and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition, and the rate of conversion of pyruvate to lactate is decreased.LDH-1 (4H) - in the heart and red blood cells,LDH-2 (3H1M) - in the reticuloendothelial system,LDH-3 (2H2M) - in the lungs,LDH-4 (1H3M) - in the kidneys, placenta, and pancreas,LDH-5 (4M) - in the liver and striated muscle. Usually LDH-2 is the predominant form in the serum. A LDH-1 level higher than the LDH-2 level (a "flipped pattern") suggests myocardial infarction (damage to heart tissues releases heart LDH, which is rich in LDH-1, into the bloodstream). The use of this phenomenon to diagnose infarction has been largely superseded by the use of Troponin I or T measurement.

Myoglobin: A hemoprotein that receives oxygen from hemoglobin and stores it in the tissues until needed. Myoglobin is a protein in heart and skeletal muscles. When you exercise, your muscles use up any available oxygen. Myoglobin has oxygen attached to it, which provides extra oxygen for the muscles to keep at a high level of activity for a longer period of time. When muscle is damaged, Myoglobin is

released into the bloodstream. The kidneys help remove Myoglobin from the body into the urine. In large amounts, Myoglobin can damage the kidneys. Myoglobin may be ordered as a cardiac biomarker, along with Troponin, to help diagnose or rule out a heart attack. Levels of Myoglobin start to rise within 2-3 hours of a heart attack or other muscle injury, reach their highest levels within 8-12 hours, and generally fall back to normal within one day. An increase in Myoglobin is detectable sooner than Troponin, but it is not as specific for heart damage and it will not stay elevated as long as Troponin. Although a negative Myoglobin result effectively rules out a heart attack, a positive result must be confirmed by testing for Troponin. Sometimes, a urine test is ordered to evaluate Myoglobin concentrations in those who have had extensive damage to their skeletal muscles (rhabdomyolysis). Blood levels of Myoglobin can rise very quickly with severe muscle injury. Urine Myoglobin concentrations reflect the degree of muscle injury and, since Myoglobin is toxic to the kidneys, reflect the risk of kidney damage. Myoglobin is not widely used for diagnosing heart attacks because it has largely been replaced by Troponin, which is much more specific. If the Myoglobin test is available, it may be ordered to assess persons with chest pain who are suspected of having a heart attack. Blood samples are drawn on admission and every 2-3 hours for up to 12 hours in those who come to the emergency room with a possible heart attack. Urine Myoglobin may be ordered when there has been extensive injury to skeletal muscle, resulting in the rapid breakdown of muscle, and damage to the kidneys is suspected. An increase in blood Myoglobin means that there has been very recent injury to the heart or skeletal muscle tissue. Additional tests, such as Troponin, are necessary to determine where the damage has occurred. Because Myoglobin is also found in skeletal muscles, increased levels can occur in people who have accidents, seizures, surgery, or any muscle disease, such as muscular dystrophy. If Myoglobin does not increase within 12 hours following the onset of chest pain, a heart attack is very unlikely. Myoglobin levels are normally very low or not detectable in the urine. High levels of urine Myoglobin indicate an increased risk for kidney damage and failure. Additional tests, such as BUN, creatinine, and urinalysis, are done to monitor kidney function in these patients. Increased Myoglobin levels can occur after muscle injections or strenuous exercise. Because the kidneys remove Myoglobin from the blood, Myoglobin levels may be high in people whose kidneys are failing. Heavy alcohol consumption and certain drugs can also cause muscle injury and increase Myoglobin in blood. A urine dipstick test for hemoglobin can also be positive in the presence of Myoglobin. If the urine dipstick test is positive and Myoglobin is suspected to be the cause, it should be followed up with more specific testing for Myoglobin[19].

Creatine Kinase (Ck): The enzyme Creatine kinase is responsible for transferring a phosphate group from ATP to creatine. Creatine kinase is composed of M and/or B subunits and forms: CK-MM, CK-MB, and CK-BB isoenzymes. CK-MB may be regarded as a sensitive and specific marker for myocardial infarction. Its value changes in 3 to 4 hours after an MI, peaks in 10–24 hours. The normal value is restored within 72 hours. Because of this short duration it cannot be used for late diagnosis of acute MI. It is however important to note that CK-MB levels also rises in conditions like: Skeletal muscle damage, renal failure. In these cases the CK index may be used. MB is divided by total CK. Measurement of CK-MB is by two methods: electrophoresis and immunoassays. Immunoassays offer better analytical sensitivity and better precision. Another utility of CK-MB is to access the success of a thrombolytic therapy. Different molecular forms of MB are found in the circulation. By checking the prevalent form of MB in the blood, one can say if the thrombolysis (breaking up and dissolving blood clots) has succeeded. It helps in determining if you have had a heart attack or if other muscles in your body have been damaged. Test for CK is done in signs and symptoms of a heart attack (e.g., chest pain); if you have muscle pain or muscular weakness. A blood

sample drawn from a vein in the arm. Creatine kinase is an enzyme found in the heart, brain, skeletal muscle, and other tissues. Enzymes are proteins that help cells to perform their normal functions. In muscle and heart cells, most of this energy is used when muscles contract[20].

Multimarker Test: The National Academy of Clinical Biochemistry (NACB) has recommended that the protocol for using cardiac markers in evaluation of patients with possible ACS should include an 'early' marker such as myoglobin or CK-MB, which is reliably increased in the blood within 6 h after symptom onset, and a 'definitive' marker (such as cTnI or cTnT), which is increased in the blood after 6–9 h with a high sensitivity and specificity for myocardial injury and remains abnormal for several days thereafter. The use of two markers is recommended due to the varying interval between chest pain onset and ED presentation. Example: chest pain patients without ST-segment elevation were examined, the use of a multimarker panel (myoglobin-CKMB-cTnI, CKMB-cTnI) identified positive patients earlier and provided better risk stratification for mortality than a single-marker (CK-MB) approach. Additionally, simultaneous determination of platelet (e.g., P-selectin) and necrosis (e.g., myoglobin, CK-MB, cTnI) markers improves the early diagnosis of AMI and congestive heart failure among the chest pain patients presenting into the ED[21].

Point-of-care Testing of Cardiac Markers: In addition to the continuous search for new biomarkers, implementation of point-of-care testing provides a convenient means for achieving early and quick detection of AMI. By doing tests in close vicinity to the patients while reaching results within minutes, POC testing for cardiac markers provides quick, accurate, and precise diagnosis so that appropriate decisions and treatment strategies can be applied in time. Both qualitative and quantitative POC testing devices have been commercially available for myoglobin, CK-MB, cTnI, and cTnT, many of which are in multimarker formats. In fact, the Stratus CS cTnI assay (Dade Behring) is the first troponin test that follows the strict guidelines suggested for troponin measurements and the Triage cardiac system (Biosite Inc.) offers an analysis panel combining not only the conventional cardiac markers but also other biomarkers such as BNP and d-dimer[21].

Device: The RAMP® system recently launched by Response Biomedical Corp., Canada is another POC immunotesting system, providing quantitative measurements of cardiac markers such as cTnI, CK-MB, and Myoglobin in whole blood. This immunochromatographic test system consists of a portable fluorescence analyzer (the RAMP reader) and a disposable test cartridge.

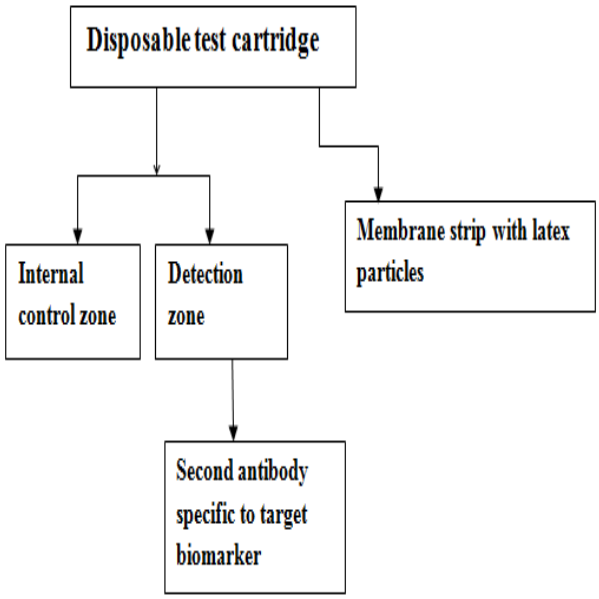

Figure 3: RAMP

Table 1: List of Cardiac Marker Device Manufacturers.

Manufacturer	Test	Cardiac marker	Quantitative or qualitative	Run time	Cardiac reader	Specimen
Abbott	i-STAT cTnI assay	cTnI	Quantitative	10 min	i-STAT® 1 handheld analyzer	Whole blood (heparinized)
Response Biomedical	RAMP system	Myoglobin	Quantitative	10–12 min	RAMP Reader®	Whole blood (EDTA)
Biosite Inc.	Triage® CardiacPanel	Myoglobin	Quantitative	15 min	Triage MeterPlus	Whole blood

The membrane strip in the cartridge contains latex particles that are fluorescently labeled and tagged with antibodies against the cardiac marker of interest. There are two special zones on the test cartridge: an Internal Control Zone and a Detection Zone that contains a second antibody specific to the target biomarker. The test cartridge is inserted into the automatic analyzer when the blood sample is applied to it. The sample moves along the strip while the target cardiac marker, if present, is bound to the antibodies attached on the fluorescent-dyed latex particles. These antigen–antibody latex complexes are captured by the antibodies on the Detection Zone, whereas the latex particles that are not bound with the target marker are arrested at the Internal Control Zone. Within less than 15 min, the RAMP reader reports a quantitative result by measuring the fluorescence emitted by the complexes captured at both zones.[20] Development of POC assays for the new biomarkers is also a big challenge. So far, the commercial cardiac marker POCT devices have been focused on the detection of myoglobin, CK-MB, and cardiac troponins. It is extremely important to develop new POC approaches for the earlier markers such as H-FABP, IMA, MPO, and sCD40L. These markers appear earlier than the normal necrosis markers and need to be tested quickly after sampling in order to give earlier indication of myocardial ischemia and/or myocardial infarction[21].

New cardiac markers under investigation: Despite the success of the current cardiac markers, there is always a need to discover and develop new biomarkers for diagnosis and management of AMI. Two principal clinical uses are targeted: early diagnosis, and risk stratification for short-term/long-term adverse events. Generally, the search for biomarkers follows two approaches. The traditional strategy is to identify the biomarkers that participate in the etiology of the disease. In the case of AMI as stated earlier, the whole physiopathology involves atherosclerosis, plaque instability, inflammation, and plaque rupture leading to platelet activation, thrombus formation, reduced blood flow, myocardial ischemia, and myocardial necrosis. The biomarkers mentioned above indicate necrosis, but other markers that reflect earlier components of the physiopathology are expected to provide independent and very useful information in AMI patients. Another approach towards biomarker discovery is to use the proteomic technique. For instance, evaluation of the NMR spectroscopy of human blood plasma led to the identification of choline as a promising marker for ACS.

Pregnancy-associated plasma protein A (PAPP-A) is a metalloproteinase originally identified in the plasma of pregnant women. It is expressed abundantly in eroded and ruptured plaques and minimally in stable plaques. As a marker of plaque instability, PAPP-A has shown its strong potential as an independent predictor of cardiovascular events in patients with ACS[21]. **P-selectin**, a membrane glycoprotein. Following platelet activation, P-selectin is rapidly expressed on the platelet surface and then rapidly cleaved off to the circulation in the soluble form. The level of the soluble P-selectin starts to rise 1–2 h after the onset of chest pain and may remain elevated for prolonged periods of time Both membrane and soluble P-selectins may serve as early markers for thrombosis-induced impending AMI, while the necrosis markers (such as cTnI and CK-MB) appear much later in the circulation and may take up to 24 h to peak following the symptom onset. A study conducted by Hollander et al. however, presented a negative observation for AMI detection in that both soluble and membrane P-selectin showed a lower sensitivity and specificity than CK-MB.

Soluble CD40 ligand (sCD40L) is another biomarker indicative of platelet activation but showing more encouraging results. CD40 ligand is a transmembrane protein expressed on platelets. Upon platelet activation, it is rapidly released to generate a soluble fragment in circulation. The CAPTURE trial with 1265 enrolled ACS patients has demonstrated the strong correlation between platelet activation and sCD40L levels, and the convincing predictive value of sCD40L in indicating the risk of cardiac events. It also demonstrated that the anti-platelet treatment using the glycoprotein IIb/IIIa receptor antagonist abciximab is beneficial to patients with elevated sCD40L levels[21].

Heart-type fatty acid binding protein (H-FABP), similar to myoglobin, is a low-molecular-weight protein (15 kDa) which is released rapidly into circulation after myocardial damage. Like myoglobin, H-FABP can be used as an early marker for acute myocardial injury, with the same kinetics of release into the blood stream. A comparison in the diagnostic performance of plasma H-FABP and myoglobin in detection of AMI reveals that H-FABP performs more effectively than myoglobin in terms of sensitivity and specificity. Moreover, H-FABP has also shown strong potential in its rule out power. Furthermore, it could be used to detect minor myocardial injury in heart failure and unstable angina, to estimate the infarct size, to judge the success of coronary reperfusion in AMI patients, and to provide an early detection of postoperative myocardial tissue loss in patients undergoing coronary bypass surgery. Therefore, H-FABP seems promising as a myoglobin substitute for improving early AMI diagnosis[21,22].

Ischemia modified albumin (IMA) has been identified as a new biochemical marker of myocardial ischemia in the early diagnosis of ACS. It is believed that the metal-binding capacity of human albumin for cobalt is lowered because of myocardial ischemia. This has led to the development of the FDA-cleared spectrophotometric albumin cobalt binding (ACB) test, which has been commonly used in clinical laboratories for quantitatively measuring IMA in human serum. Quite a few clinical studies regarding the evaluation of IMA and the ACB test have been conducted, one of which was a study assessing 200 ED patients with suspected myocardial ischemia with IMA and standard necrosis biomarkers. The ACB test yielded a sensitivity of 80%, while the Myoglobin-CK-MB-cTnI triad and the combination of IMA-Myoglobin-CK-MB-cTnI showed a sensitivity of 57% and 97%, respectively. This clearly suggests that IMA is highly sensitive in the diagnosis of myocardial ischemia, especially when used in combination with those currently used standard cardiac markers. Similar results were reported in However, there still remain some questions concerning both IMA and the ACB test, which require additional clinical evidence for support[21].

Small Molecules as Biomarkers

Some smaller molecules present in blood have also been found to play important roles in indicating AMI. For example, the connection of the changes in serum homocysteine concentration to AMI has led to the suggestion that the increase in serum homocysteine concentration may be taken as one of the risk-factors in the development of AMI. On the other hand, the relationship between trace elements (such as Cu, Fe, Zn, Se) and cardiac markers in acute coronary syndromes has also implied that serum levels of these trace elements may be related to the degree of myocardial damage. The number of patients suffering from HF is increasing rapidly and, on a global scale, the majority will not be cared for by highly specialized medical centers. This systematic review will, hopefully, help in the development of widely applicable management strategies. At present, BNP and NT-proBNP are the biochemical markers, which make the greatest contributions in the (1) risk stratification, and (2) diagnosis of HF. Several other emerging biochemical markers are being investigated, and study protocols based on multiple markers with a view to risk stratify and monitor or target therapy warrant consideration[21].

CONCLUSION

Future studies will continue to concentrate on searching for new specific biomarkers of cardiac injury and on the development of new sensitive POC assays for cardiac markers. The main focus will still be on method standardization and improvement in analytical sensitivity and specificity. Harmony between the results obtained in central laboratory and those obtained by various POC assays remains an important issue. The number of patients suffering from HF is increasing rapidly and, on a global scale, the majority will not be cared for by highly specialized medical centers. This systematic review will, hopefully, help in the development of widely applicable management strategies. At present, BNP and NT-proBNP are the biochemical markers, which make the greatest contributions in the (1) risk stratification, and (2) diagnosis of HF. Several other emerging biochemical markers are being investigated, and study protocols based on multiple markers with a view to risk stratify and monitor or target therapy warrant consideration.

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