A REVIEW ON PATHOLOGY OF MYOCARDIAL ISCHEMIA AND VARIOUS TYPES OF NOVEL BIOMARKERS

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ABSTRACT

Ischemia can also be described as an inadequate flow of blood to a part of the body, caused by constriction or blockage of the blood vessels supplying it. Ischemia of heart muscle produces angina pectoris. Since oxygen is mainly bound to hemoglobin in red blood cells, insufficient blood supply causes tissue to become hypoxic, or, if no oxygen is supplied at all, anoxic. In very aerobic tissues such as heart and brain, at body temperature necrosis due to ischemia usually takes about 3-4 hours before becoming irreversible. This and typically some collateral circulation to the ischemic area accounts for the efficacy of "clot-buster" drugs such as Alteplase, given for stroke and heart attack within this time period. However, complete cessation of oxygenation of such organs for more than 20 minutes typically results in irreversible damage. In many cases, obstruction of the coronary artery by thrombus is minimal, resulting in little or no impairment to coronary flow. Alternatively, the thrombus can result in total occlusion of the artery with classic symptoms and ECG findings. The combination of reduced blood flow and increased oxygen demand precipitates the critical imbalance of oxygen supply and demand that leads to myocardial ischemia (i.e., unstable angina). Persistent ischemia can result in myocyte death, which may be detected using biomarkers of necrosis and forms the basis for the diagnosis of AM

Keywords: Ischemia, clot-buster, atherothrombotic, biomarkers, necrosis

INTRODUCTION

This major limitation could be overcome if ischemia could be reliably detected biochemically. A simple assay with high sensitivity and specificity for cardiac ischemia and a kinetic profile useful for an acute event marker would greatly simplify and improve the safety of the chest pain evaluation process. Biochemical tests tend to be relatively inexpensive and practical to perform, thus allowing widespread application. They can generally be performed with rapid turnaround, providing support for clinical decision making. Understanding the pathophysiology of ACS, the biochemical changes that occur, and the attendant clinical imperatives has fostered a concerted effort aimed at identifying biochemical markers of ischemia that can be widely applied toward the evaluation of patients with possible ACS. All of these characteristics provide a strong argument toward development of biochemical markers of myocardial ischemia. Nevertheless, there are many challenges to this effort [1].

The surface 12-lead ECG, along with perfusion and functional imaging, provides proof of principle that ischemia can be detected early in the course of ACS. Furthermore, the absence of ischemia using this approach indicates low risk and allows safe discharge, while the rapid detection of ischemia provides an opportunity for early intervention that can improve outcomes, especially if necrosis has not yet occurred. Nevertheless, dependence on the ECG and symptoms alone is insufficiently sensitive, and strategies that include perfusion and functional imaging require a substantial investment in technology and expertise that has hindered their widespread application [2-5].

Ischemia is a feature of heart diseases, transient ischemic attacks, cerebrovascular accidents, ruptured arteriovenous malformations, and peripheral artery occlusive disease. The heart, the kidneys, and the brain are among the organs that are the most sensitive to inadequate blood supply. Ischemia in brain tissue, for example due to stroke or heavy injury, causes a process called the ischemic cascade to be unleashed, in which proteolytic enzymes, reactive oxygen species, and other harmful chemicals damage and may ultimately kill brain tissue [6].

Restoration of blood flow after a period of ischemia can actually be more damaging than the ischemia. Reintroduction of oxygen causes a greater production of damaging free radicals, resulting in reperfusion injury. With reperfusion injury, necrosis can be greatly accelerated. Low doses of hydrogen sulfide (H_2S) have been found to protect against regional myocardial ischemia–reperfusion injury [6].
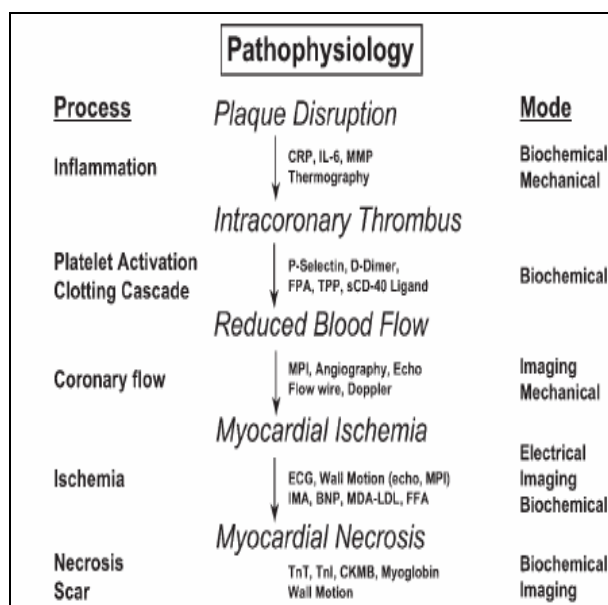

Figure 1: Mode/ Mechanism of Action and Targets for detection of ACSs

Applicable Various Novel Biomarkers of Myocardial Ischemia

Myocardial ischemia has a complex pathophysiology and can manifest with a multitude of clinical presentations, including without symptoms. Patients who present to the emergency department with a complaint of chest pain therefore require substantial diagnostic effort to determine whether their symptoms are related to acute myocardial ischemia. Present diagnostic approaches are typically protocol driven and include serial electrocardiograms and biomarkers obtained over an 8- to 12-h period for the detection of myocardial necrosis. This paradigm is reasonably effective in identifying patients who have acute coronary syndrome, especially when there is associated myocardial necrosis, but it offers only modest sensitivity and relies heavily on biomarkers that are increased only in the presence of irreversible myocardial injury (i.e., necrosis). For this reason, there has been intense research and clinical interest in the development of sensitive biomarkers of myocardial ischemia that are not dependent on the presence of myocardial necrosis [7]. Markers investigated for this purpose include ultrasensitive troponin, ischemia-modified albumin, sCD40L, myeloperoxidase. Despite the emergence of these candidate markers of ischemia, currently there is not sufficient evidence to recommend widespread adoption of any of them into clinical practice.

Ischemia-Modified Albumin [IMA] [8]

In 2003, IMA became the first FDA-approved ischemic biomarker and was recommended for use in ruling out ACS, especially in patients presenting with typical acute chest pain but normal or non-diagnostic ECGs. The basis for this test, developed by Ischemia Technologies, lies in the discovery by Bar-Or et al., that albumin in the serum of patients with myocardial ischemia exhibited lower metal-binding capacity for cobalt than the albumin in serum of normal subjects. The 'Albumin Cobalt Binding' (ACB™) Test for IMA (Ischemia Technologies, Inc., Denver, CO) monitors the binding between serum albumin and the transition metal cobalt. This change in cobalt binding capacity was found to become significant only minutes after transient ischemia induced by balloon angioplasty, returning to normal within 12 h. Initially, the prospects of IMA looked promising with one study by Christenson et al. demonstrating that IMA levels exhibited a high negative predictive value and sensitivity for predicting cardiac cTnI-negative or cTnI-positive results 6–24 h later. Furthermore, it was reported that the combination of the IMA assay with traditional biomarkers like myoglobin, CK-MB and cTnI, increased the diagnostic sensitivity for myocardial ischemia from 57% to as much as 97%. On the other hand, these ischemic-related increases in IMA may be due to nonspecific injury of tissues other than the myocardium. Modifications to the N-terminal regions of albumin may be induced by mechanisms such as endothelial and extracellular hypoxia, acidosis, free radical injury and sodium/calcium pump disruptions. However, it has also been observed that many patients without evidence of ischemia can have elevated levels of IMA. For instance, there have been reports of raised IMA levels following vigorous aerobic training,

radiofrequency catheter ablation, and skeletal muscle ischemia or in patients with peripheral vascular disease and in diseases associated with oxidative stress such as systemic sclerosis. In addition, a deletion defect in the N-terminus of albumin was documented in a non-ischemic individual, which masked the decreasing cobalt-binding effect of cardiac ischemia. Furthermore, Worster et al. found that an IMA assay drawn at the time of patient presentation was only 70% sensitive and 24% specific for serious cardiac outcomes. Ischemia Technologies was acquired by Inverness Medical Innovations, Inc. and in 2006 proceeded to market its Albumin Cobalt Binding (ACB®) test for detection of ischemia modified Albumin (IMA®) on the Roche Diagnostics Corporation Cobas Integra® 700 and Cobas Integra® 800 chemistry analyser systems.

Unbound free fatty acids [UFFA] [4-9]

Recently, FFAu has generated much interest as a biomarker of cardiac ischemia. One study in particular reported significant increases of the biomarker in ischemic-related events. Serum FFAu concentrations are determined from the ratio of total serum free-fatty acids (FFAs) to total serum albumin. Several mechanisms have been proposed for the role FFAs play during ischemia. A study by Hendrickson et al. investigated the effect FFA inhibitors had on the progression of cardiac events and noted a reduction in infarct size and a decrease in the post ischemic cardiac dysfunction in animal models. Two studies documented the occurrence of FFAu elevations well in advance of other, more traditional, biomarkers of cardiac necrosis. The Paris Prospective Study 1 involved a cohort of 5250 middle-aged men, free of known ischemic cardiac disease. Initial FFA concentrations and FFA concentrations after a 22 year follow-up were measured leading to the conclusion that an increase in FFA was an independent risk factor for sudden death but not for fatal MI. However, there is the possibility that heparin may cause FFA increases in vivo and this may lead to non-specific FFAu levels. Unlike the commonly applied sandwich-based immunoassay format used for detection of FFAu, a novel hand-held device by FFA Sciences LLC for FFAu measurement is available, requiring only 15 ìL of plasma with a turnaround-time of b1 min. The FFAu is measured with a recombinant fatty acid-binding protein labelled with a fluorescent tag (ADIFAB2). When FFA binds to ADIFAB2, the fluorescent tag is displaced from its binding pocket, producing a spectral shift that can be measured with a fluorimeter. The assay has shown improved low-end sensitivity compared to the first generation ADIFAB assay. Cohort trials involving a broad spectrum of suspected myocardial ischemia patients need to be performed to fully evaluate the potential usefulness of FFAu.

C-reactive protein [10]

CRP is an acute phase reactant, produced in the liver in response to interleukin (IL)-6. It is a highly stable pentameric protein and its serum concentration can increase 10,000-fold during the acute phase response. Investigative data suggests that CRP may even play an active role in atherogenesis. It has been shown that CRP co-localizes with the membrane attack complex in early

atheromatous lesions. Two more recent studies reported the detection of CRP in the early stages of plaque development and also stated a possible involvement in facilitating processes ranging from the initial recruitment of leukocytes to the arterial wall to the eventual rupture of the plaque itself. Another study observed the loss of the pentameric symmetry of CRP resulting in a modified or monomeric CRP form (mCRP), which may be the major CRP isoform promoting the proinflammatory response in coronary arteries. In January 2003 both the Centres for Disease Control and Prevention (CDC) and the American Heart Association (AHA) recommended CRP as the inflammatory biomarker of choice for assessment of cardiovascular risk. The high sensitivity CRP assay (hs-CRP) was originally developed to aid evaluation of conditions associated with inflammation, in otherwise healthy individuals. Cardiac CRP (cCRP) assays are indicated for use in the clinical identification and stratification of patients at risk of future CVD-associated events. The hs-CRP assay is frequently quoted in a cardiac context and indeed both hs- CRP and cCRP assays have equivalent clinical cut-off and measuring ranges. Nonetheless, the additional performance validation demanded to support the expanded intended use of CRP in indications related to CVD dictates that a clear distinction be made. The recommended guidelines for primary prevention set cut-off points according to relative risk categories: low risk (b1 mg/L), average risk (1–3 mg/L) and high risk (N3 mg/L) for patients with an intermediate 10-year CVD risk (10% to 20% Framingham Risk Score/ATP III guidelines). Recently, hs-CRP has also been incorporated into a cardiac risk score for women, the 'Reynolds risk score', based on a comprehensive study involving nearly 25,000 women over 10 years. Several large-scale epidemiological studies examining apparently healthy populations have found CRP to be a strong independent predictor of future cardiovascular events, including MI, ischemic stroke, peripheral vascular disease and sudden cardiac death. This is evident in the growing number of commercially available hs-CRP tests, one example being the Pathfast® POC test supplied by Mitsubishi Chemical Europe GmbH.

Cardiac Troponins [11]

Both cTnI and cTnT are highly utilised specific biomarkers for myocardial tissue. Their detection in serum is a strong indicator for myocardial damage and, consequently, both have shown great potential as cardiac biomarkers. Several international scientific bodies e.g. the European Society of Cardiology (ESC), the American College of Cardiology (ACC), the American Heart Association (AHA) and the National Academy of Clinical Biochemistry (NACB) have recommended the use of these biomarkers when implementing new diagnostic strategies. The cardiac troponins were also key components in the comprehensive re-evaluation of the definition of MI. Moreover, the significant prognostic potential of the cardiac troponin family (TnI and TnT) was independently confirmed in clinical trials (TACTICS-TIMI 18, FRISC and OPUS-TIMI). Conversely, it was discovered that only 22–50% of patients with unstable angina have positive cardiac troponin (I or T) tests. While initially selected for their high specificity, elevated cardiac troponins have been shown to have possible links with secondary conditions such as chronic renal failure, acute myocarditis, cardiomyopathy, congestive heart failure (CHF), pulmonary embolism (PE), rhabdomyolysis, sepsis and left ventricular hypertrophy. Such non-specific issues, however, were associated with the first generation cTnI and cTnT assays. Recently, the analytical performance of cardiac troponin assays has improved significantly compared to their initial clinical implementation. For example, early generation cTnI assays were compromised by a variety of factors. Serum interference from patient heterophile and human anti-species antibodies contributed to false positive results and the presence of autoantibodies and varying forms of cTnI (e.g. truncated or complexed with TnC/TnT), masked diagnostic antibody-binding epitopes. Much effort has been invested in defining the most suitable target epitopes within the cTnI molecule for maximising cardiac specificity and assay sensitivity. The current array of cTnI assays are highly cardiac-specific and this was achieved by targeting the cardiac specific N-terminal region (amino acids 41–49, in particular) in two- or three-site sandwich immunoassay formats. Notwithstanding these improvements, there remained severe impediments to standardizing cTnI assays, due ostensibly to the lack of a suitable reference material. Purified cTnI contains urea and EDTA and thus, can undergo carbamylation and is not representative of the circulating troponin I/T and I/T/C complexes or troponin degradation products. A primary reference material for cTnI is available. However, despite harmonisation efforts, studies indicate that cTnI assays remain non-standardized after recalibration using this material. To achieve closer comparability of catnip values between assays, the use of a secondary reference material, consisting of a panel of human serum pools, is proposed for use by manufacturers to calibrate their assays. Considerable attention must be given to the newly established practical guidelines addressing implementation of such cardiac biomarkers, prior to adoption of these assays. It must be acknowledged, however, that cardiac troponin results should be supplementary to and not a replacement for, informed clinical decision making. The recent emergence of high sensitivity hs-cTnI assays is postulated to facilitate earlier detection of myocardial injury comparable with detectable levels of cTnT. As sensitivity is improved, the precise cut-off determinants become increasingly important. The IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory recommends that the 99th percentile of the reference population should be the decision limit for myocardial injury. A number of recent commentaries have, however, indicated clear weaknesses in trying to implement a blanket 99th percentile cut-off. Such discrepancies raise the question as to whether healthy individuals can be better characterized for use in determining reference limits. Currently, there is no consensus definition of what constitutes a hs-cTnI test and, with the predicted evolution of newer more sensitive assay config categorise 'cut-offs' based on age, gender or ethnicity. In fact, there is now a clear movement towards ultrasensitive detection formats demonstrating sensitivityb1 ng/mL and, thus, serial testing is most definitely needed to gauge low level population variation. Moreover, this could herald the beginning of a move

towards personalized cardiac healthcare and this would obviate the need for standardized cut-offs as individual relative rising levels are independently monitored. The troponins (cTnI and cTnT) remain key biomarkers for patient triage in the ED due to their accuracy in identifying low risk PE patients and high negative predictive value for in hospital death. Another biomarker family which shares these characteristics is the B-type natriuretic peptides (BNP) and N terminal pro-B-type natriuretic peptide (NT-proBNP), which are also routinely used in the ED. These peptides are synthesized in the cardiac ventricles and belong to a family of naturally occurring hormones known as natriuretic peptides. These peptides are produced in increased amounts by the heart in response to CHF and function by lowering pressure in the lungs and also by increasing urine flow. The considerable day to day variation for both BNP and NT-proBNP requires high delta change values for evaluating patients for statistical significance.

Myloperoxidase [12]

MPO is a mediator enzyme secreted by inflammatory cells e.g. activated neutrophils and monocytes and is found in atherosclerotic plaques. Accumulating evidence indicates that MPO may also have a causative role in plaque vulnerability. MPO can generate several reactive oxidized intermediates, concomitantly inducing oxidative damage of cells and tissues. Such reactive intermediates have been found at increased levels in low density lipoprotein (LDL) isolated from atherosclerotic lesions. Moreover, high MPO expression levels have been reported in patients with sudden cardiac death, at sites of plaque rupture, superficial erosions and in the lipid core, whereas less harmful fatty streaks were observed to exhibit little MPO expression. Several recent clinical studies have suggested that MPO levels may play a potentially important diagnostic and prognostic role in endothelial function, CAD and ACS. The role of MPO as an independent predictor of endothelial dysfunction has been demonstrated and further studies have underlined the value of MPO as a predictor of cardiac risk in populations with different incidences of ACS. Both studies concluded that a single measurement of plasma MPO at hospital admission predicted the risk of major adverse cardiac events for both a 30-day and six month period. Baseline levels of MPO were determinants of patient risk stratification, even in patients with consistently negative cTnT levels. The predictive power of MPO was also found to be independent of soluble fragment CD40 ligand (sCD40L), CRP and BNP in ACS patients with undetectable cTnT concentrations and sCD40L concentrations below their threshold values. MPO levels, in contrast to cTnT, CK-MB and CRP levels, identified patients at risk for cardiac events in the absence of myocardial necrosis. It has subsequently been suggested that MPO may play an important role in identifying short-term risk of recurrent ischemic events, again, independent of clinical risk factors like cTnI and other novel biomarkers. A case control study highlighted that increasing levels of leukocyte-MPO and blood-MPO were significant predictors of CAD. Individuals in the highest quartile of blood-MPO had a 20-fold higher risk of CAD than individuals in the lowest quartile, following adjustment for white blood cell count and Framingham

risk score. In August 2005, the FDA approved the first MPO assay, Cardio MPO™, developed by Prognosti X, Inc. The test is a sandwich enzyme immunoassay cleared for use in conjunction with clinical history, ECG and other cardiac biomarkers to evaluate patients with chest pain at risk of major adverse cardiac events. MPO appears to be one of the most promising contemporary candidates, yet due to its relatively recent emergence, there is still uncertainty about the additional benefits of MPO over standard ischemic biomarkers. Therefore, further supporting evidence is required to clarify this issue before it achieves widespread clinical acceptance. Moreover, almost all data for MPO is in the setting of ACS or established CAD and, thus, it remains unclear whether MPO can provide additional information to conventional atherothrombotic risk factors used in the Framingham Risk Score.

Natriuretic peptides [13]

B-type natriuretic peptide (BNP) is a neurohormone secrete from the cardiac ventricles as a response to pressure or volume overload. B-type natriuretic peptide, an established cardiac biomarker for patients with heart failure, is also elevated in patients with ACS and can identify ACS patients who are at higher risk for adverse cardiovascular events, including heart failure or death. The utility of BNP as a diagnostic cardiac biomarker for ACS is based upon the premise that early after the onset of ischemia, impaired diastolic (and often systolic) function occurs with subsequent increases in left ventricular pressure and volume overload, potentially resulting in release of BNP. As the half life of BNP is approximately 20 minutes, dynamic changes in BNP can occur rapidly. B-type natriuretic peptide levels rapidly decrease in unstable angina patients with resolution of ischemia. This suggests that BNP may provide important diagnostic and prognostic information in patients with potential ACS, particularly in the undifferentiated ED population.

Soluble fragment CD40 legends (sCD40L) [14]

sCD40 legends are a signaling protein that reflects both inflammatory and platelet interaction. It was reported that elevations of sCD40L correlated with an increased risk of cardiac events during six months of follow-up. Another study observed that in patients who were negative for myocardial necrosis, sCD40L indicated a sub-group at increased cardiac risk. However, a rise in sCD40L was associated with many other inflammatory conditions i.e. rheumatoid arthritis, sickle cell anemia and systemic lupus erythematosus and the derth of data to support the cardiac-specific clinical value of sCD40L dictates that further evaluation is warranted. Furthermore, several preanalytical parameters such as the anti-coagulant used, centrifugation speed, temperature and time to analysis have been identified as important factors with regard to measurement of sCD40L, which may prove to be another potential barrier to the practical application of this biomarker.

CONCLUSION

In many cases, obstruction of the coronary artery by thrombus is minimal, resulting in little or no impairment to coronary flow. Alternatively, the thrombus can result in

total occlusion of the artery with classic symptoms and ECG findings. The combination of reduced blood flow and increased oxygen demand precipitates the critical imbalance of oxygen supply and demand that leads to myocardial ischemia (i.e., unstable angina).The search for an effective biomarker of myocardial ischemia remains a high scientific priority. Despite the emergence of multiple candidate markers for the detection of ischemia and/or diagnosis of ACS, as yet, none of these has sufficient evidence to recommend widespread adoption into clinical practice.

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