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REVIEW

Cardiac Markers in the Early Diagnosis and Management of Patients with Acute Coronary Syndrome

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استخدام العلامات القلبية الواصمة في التشخيص والعلاج المبكر للمرضى الذين يعانون من المتلازمة القلبية الحادة

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الملخص: يعد ألم الصدر شكوى غير نوعية على الرغم من أنه غالبا ما يكون السبب الأكثر حدوثا الذي يجبر المريض على طلب الرعاية الطبية. مجموعة صغيرة من هؤلاء المرضى يعانون من المتلازمة القلبية الحادة. نظام التشخيص المتبع حاليا والمبني على التاريخ السريري وتخطيط القلب غير كافي. وقد يؤدي في بعض الأحيان إلى التشخيص الخاطئ وإدخال المرضى إلى الأقسام غير المناسبة. أو أن يتلقوا رعاية أو أدوية أو فحوصًا غير مناسبة. وفي بعض المرضى يتأخر التشخيص وهذا يؤدي إلى تأخير إعطاء الأدوية الضرورية أو حتى عدم إعطائها. قليل من المرضى الذين يعانون من المتلازمة القلبية الحادة قد يتم إخراجهم من الطوارئ خطأ. مما قد يترتب عليه أخطار صحية وقانونية. كما أن نظام التشخيص هذا قد يؤدي أيضا إلى إدخال عدد كبير من المرضى الذين لا يعانون من أمراض قلبية إلى المستشفى بدون داع. يمكن تطوير النظام الحالي لتشخيص وعلاج المرضى الذين يعانون من آلام الصدر بشكل كبير عن طريق إدخال نظام فحص العلامات القلبية الواصمة المتسلسلة للكشف المبكر جدا عن الجلطة القلبية الحادة. هذا المقال يتناول العلامات القلبية الواصمة المتوفرة حاليا لتشخيص تلف عضلة القلب مثل كرياتين كاينيز. كرياتين كاينيز العضلة والدماغ (كتلة و فاعلية). كرياتين كاينيز العضلة والدماغ (مثلي). البروتين الرابط لحمض القلب الدهني. مايوجلوبين. تروبنين القلب (ت) وتروبنين القلب (أ).

مفتاح الكلمات: العلامات القلبية الواصمة. المتلازمة القلبية الحادة. جلطة قلبية حادة. ألم الصدر.

ABSTRACT: Chest pain is a non-specific complaint and is the most frequent reason for patients seeking urgent medical attention. A small group of these patients will have acute coronary syndromes (ACS). The current diagnostic and triage systems based on clinical history and electrocardiograms are insufficient. They may result in some of these patients being misdiagnosed and being admitted to the wrong units or receiving inappropriate care, treatment and investigations. In some patients, the diagnosis is delayed resulting in the late administration (or no administration) of essential early treatment. A few patients with ACS may be inadvertently discharged from the emergency department leading to serious health and legal implications. These systems also result in the unnecessary admission of a substantial number of patients without ACS. The triage and management of patients with chest pain can be considerably improved by implementation of serial cardiac markers testing that can identify ACS in the very early stages of presentation. This review article will discuss the currently available markers of myocardial damage such as creatine kinase (CK), creatine kinase muscle and brain (CK-MB) (mass and activity), CK-MB isoforms, heart-type fatty acid-binding protein, myoglobin, cardiac troponin T, and cardiac troponin I.

Keywords: Cardiac markers; Acute coronary syndromes; ACS; Acute myocardial infarction; AMI; Non-ST elevation myocardial infarction; NSTEMI; Chest pain.

Acute myocardial infarction (AMI) is the leading cause of death in developed countries;[1] however, in-hospital mortality from this cause has been declining over the last three decades.[2] This reduction in mortality coincides with the improvement in health and living standards and with new treatments like thrombolysis and new interventions like percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG). Secondary and primary prevention strategies have contributed significantly and the age-adjusted mortality is expected to continue to decline with further improvements in treatments, better uptake of primary and secondary prevention strategies and also with further improvement in our ability to recognise this challenging disease very early in its course. The success of treatment rests on: 1) identification of patients in the very early stages of AMI; 2) implementation of treatment to recanalise the occluded artery; 3) access to early defibrillation and 4) admission to properly monitored coronary care or intensive care units (CCU or ICU) for the

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detection and treatment of complications.

The diagnosis of AMI was previously based on the criteria set by the World Health Organization (WHO) and had to include two of the following: 1) typical history of prolonged ischaemic chest pain; 2) presence of typical acute ischaemic changes on the admission electrocardiograms (ECG); 3) typical rise and fall of cardiac enzymes in blood.[3] This old definition has recently been changed with the publication of a new definition of myocardial infarction by The Joint European Society of Cardiology/American College of Cardiology (ESC/ACC) Committee, which redefines myocardial infarction (MI) according to cardiac markers as follows: 1) an increase in cardiac troponin cTnI, cTnT exceeding the decision limit (99th percentile of the value for a reference control group) on at least one occasion; 2) an increase in creatine kinase muscle and brain (CK-MB), preferably CK-MB mass exceeding the decision limit (99th percentile of the value for a reference control group) on at least two occasions with a rise and fall pattern, or greater than twice the upper limit of the reference range on one occasion. Within this definition, acute coronary syndromes (ACS) are classified into ST and Non-ST elevation. ST elevation ACS is further classified into Q-wave and non Q-wave MI. Non-ST elevation MI (with cardiac marker elevation) is also classified into Q-wave and non Q-wave MI. Non-ST elevation ACS without cardiac markers elevations is called unstable angina.[4]

The very early diagnosis of AMI can be a challenging task for many physicians in an emergency department. When a typical history is present, it helps to orient the clinician to the right diagnosis, but its absence by no means rules it out. This is often the case in a significant proportion of patients in whom the history is either atypical or absent. Diabetic, hypertensive, and elderly patients often have silent AMI. In these cases AMI may go unnoticed or may produce atypical symptoms such as hypotension, breathlessness, syncope, or arrhythmias.[5] The ECG is an important tool for detecting AMI, but it lacks sensitivity and as many as 30-50% of patients may initially present with normal or non-diagnostic ECG.[6] The ECG has an overall diagnostic sensitivity for AMI of 70-81%;[7] however, when changes typical of AMI are present on the admission ECG (ST elevation and new Q-wave) they are highly specific and have a very high positive predictive value for the diagnosis of AMI. Cardiac markers are formidable tools for the diagnosis of AMI.[8]

Characteristic Features of Biochemical Markers of Myocardial Injury

The ideal characteristics of a marker of myocardial injury are: 1) it should be abundant in the myocardium and not present in other tissues. This gives it a high specificity for the myocardium and reduces the rate of false positive results; 2) it should have a high concentration in the myocardium and a low or undetectable concentration in the blood in the absence of disease. This gives it a high sensitivity so that the release of only a small amount can be readily detected thereby reducing the rate of false negatives; 3) it should be released completely and quickly when myocardial damage occurs. This will allow its utilisation for the early detection and quantification of injury; 4) it should persist in the circulation to give a convenient diagnostic window, but not so long as to prevent the detection of complications such as early re-infarction, and 5) the assay must have a high analytical sensitivity and specificity and a short turn around time, so that results could be obtained fast enough to influence the decision-making process regarding patients' triage and management.

Biochemical Markers and Early Detection of Acute Myocardial Infarction

Cardiac markers play an important role in the detection of AMI when the patient's history and ECG are non-diagnostic or equivocal.[9] Diagnosing AMI early (i.e. within 6 hours after symptom onset) is difficult, because some time must elapse after symptom onset for markers to exceed values above the reference range. The diagnosis of AMI based only on one single value at presentation or soon after admission is unreliable, and serial sampling is the most effective method. The sensitivity and specificity of cardiac markers for the early diagnosis of AMI is influenced by several factors such as:

1. *Time of presentation:* Early presentation after symptom onset is likely to show a relatively

increased sensitivity for markers like myoglobin, which is released very early in the course of AMI, and less sensitivity for markers like CK-MB or cTnI, which are released slightly later. A delayed presentation is likely to have the opposite effect. Therefore, the onset of symptoms should be used as the reference point when commenting on the sensitivity and specificity of the markers for the early diagnosis of AMI rather than criteria such as time of presentation, and time of admission.[10]

2. *Size of infarct:* Cardiac markers are released in proportion to the volume of myocardium in jeopardy, the bigger the infarct the greater the quantity of cardiac markers released. This will lead to improved sensitivity compared to small infarcts where a limited release of markers may be close to the threshold for detection.[11]

3. *Selection criteria and prior probability of acute myocardial infarction:* Selecting certain group(s) of patients for the study with a high possibility of AMI, such as those admitted to the CCU, can also influence sensitivity of a marker.

4. *Treatment:* Treatment can have an influence on the sensitivity of the marker. For example, patients who have been successfully thrombolysed show a greater and earlier peak of some of the markers compared to those who were not successfully reperfused.[12]

5. *Diagnostic threshold:* The selection of an appropriate diagnostic threshold requires careful consideration of concentrations seen in the normal and disease free population and those seen in diseased populations. There is always a continuous balance between the sensitivity and specificity of any marker. If the cut-off concentration used is low, the sensitivity is improved at the expense of specificity unless the marker is 100% cardiac specific, and only present in diseased populations.[13]

6. *Kinetic factors:* Kinetic factors like the molecular size of the marker, the biological compartment of the marker (i.e. whether it exchanges freely in the cytoplasm or is attached to structural elements within the cell), the volume of distribution, and whether the marker is released directly into the blood or cleared by the lymphatic system can all influence plasma concentration. Markers that have a low molecular weight, lie free in the cytoplasm, and are released directly into the circulation show better early sensitivity compared with larger molecules that are attached to structural elements and/or cleared slowly by the lymphatic system.[14]

It is not surprising therefore that different studies report differing results for sensitivity and specificity for the various cardiac markers for the early diagnosis of AMI. The variations in the reported results will predominantly be influenced by the above factors. This review article will discuss the currently available markers of myocardial damage like creatine kinase (CK), CK muscle and brain (MB) (mass and activity), CK-MB isoforms, heart-type fatty acid-binding protein (H-FABP), myoglobin, cardiac troponin T (cTnT) and cTnI. The discussion will focus in particular on the suitability of these markers as tools for: 1) the early diagnosis of MI within 6 hours; 2) their ability to detect myocardial damage during ischaemic episodes in patients with Non-ST elevation MI or undergoing cardiac intervention like PCI or surgery; 3) their ability to guide further treatments i.e. antiplatelets, antithrombotics, coronary interventions, etc; 4) their ability to detect reperfusion and re-infarction; 5) their specificity and limitations and 6) future directions with these markers. For the sake of clarity, unstable angina with elevated cardiac markers quoted in various references in the literature will be used synonymously with Non-ST elevation MI using the recent new definition of acute coronary syndromes referred to above.[4]

Creatine Kinase

Creatine kinase is an enzyme composed of two subunits, M and/or B. Three different pairs of these units combine to give rise to three different isoenzymes, CK-BB, CK-MB and CK-MM. CK-BB is the brain isoenzyme and is present in large quantity in the brain and many internal organs. CK-MB is the heart specific isoenzyme and has been the gold standard method for the diagnosis of AMI in many laboratories. It exists in large quantity in heart muscle, but is not totally cardiac specific and exists also in skeletal muscles and other tissues.

About 15-40% of the total CK activity of heart muscle is due to CK-MB, the rest is largely due to the CK-MM isoenzyme. CK-MM is the skeletal muscle isoenzyme. It has the highest distribution in skeletal muscles. The three isoenzymes are present in varying concentrations in the smooth muscle of the colon, ileum, stomach, and urinary bladder.[15] The reference range for CK is approximately 80-200 IU/L for men and 60-140 IU/L for women. This is the result of the normal turnover of this enzyme in skeletal muscles, and it is influenced by factors like muscle mass and physical work.

CREATINE KINASE AND ACUTE MYOCARDIAL INFARCTION

Creatine kinase was introduced in 1965 as a biochemical marker for myocardial damage and it is one of the oldest markers in this field.[16] It has a clinical sensitivity for the diagnosis of AMI of 90%. Unfortunately, this is not matched by high specificity. It is released within 12 hours after symptom onset of AMI, peaks in serum at 24-36 hours, and returns to normal in 48-72 hours. As a result of these release kinetics, measurement of total CK is not suitable for the early diagnosis (within 6 hours) of AMI. CK as a marker is also unsuitable for the detection of myocardial damage that may occur in patients presenting with Non-ST elevation MI, or in patients undergoing PCI or surgery. As mentioned earlier, a marker that is suitable for the early diagnosis of AMI, and the detection of small injuries to the heart should have: a high cytoplasmic (cell) to vascular (plasma) ratio, very low or undetectable normal plasma concentration and total cardiac specificity. CK does not fulfil these criteria since it has a moderate cytoplasmic to vascular ratio of 60,000:1; a high reference range up to 200 IU/L, and is widely distributed in the body. To improve on the cardiac specificity of CK for the diagnosis of AMI, it was recommended to measure both total CK and CK-MB (the cardiac specific isoenzyme of CK). A CK-MB to CK ratio of > 6% is reported to be specific for myocardial injury, whereas a ratio of < 6% is consistent with skeletal muscle damage or non-cardiac causes.

In some clinical settings, a CK-MB test is not requested unless the total CK activity is elevated. The use of total CK as a screening test before ordering CK-MB could miss some patients with AMI and should be used with caution. There are cases of AMI without elevation of total CK concentration, but the total CK-MB fraction and the CK-MB to CK ratio in these patients is diagnostic for AMI. This is likely to happen in situations when there is a small MI in a small sized person with a low muscle bulk, and a low baseline value of total CK. The normal reference range of CK is very broad and these patients could release small amounts of the enzyme, insufficient to raise the concentration above the reference range. If this possibility is not borne in mind, the diagnosis could be missed when CK is used. Unless the baseline total CK is known in a patient presenting with highly suspicious diagnosis of ischaemia, a low total CK should not exclude the diagnosis nor should it preclude requests for CK-MB.[10]

LIMITATIONS AND FUTURE DIRECTION OF CREATINE KINASE MEASUREMENT

The CK enzyme is widely distributed and there are various causes of elevated total CK in the absence of myocardial injury.[17] Haemolysis leads to the release of the adenylate kinase enzyme, which interferes with the assay and causes false elevation of total CK activity. Various forms of skeletal muscle injury can lead to increased CK activity including strenuous exercise, intramuscular injection, rhabdomyolysis, burns, and trauma. Chronic muscle diseases like polymyositis, dermatomyositis, and myopathy can all lead to increased concentrations of CK. Total CK activity is a very sensitive indicator of injury to skeletal muscles. Drugs like cocaine and alcohol can also raise CK concentrations to abnormal values, presumably due to the associated myopathy that can occur with the use of these drugs. Neurological conditions like myasthenia gravis also elevate total CK activity. Other miscellaneous conditions including pregnancy, hypothermia, and sepsis can increase total CK concentrations.[18] In many of these situations, the activity of CK-MB is also increased, giving a ratio of CK-MB to CK that remains below the 6% cut-off point required to differentiate between skeletal muscle injury and cardiac muscle injury.[19] Measurement of CK is a relatively cheap assay that is widely available. For this reason, total CK measurement will probably continue to be used as a marker for myocardial injury especially in situations such as: 1) absence of cTnI, cTnT, and CK-MB assays; 2) patients with unequivocal ECG diagnosis of MI where a non-specific marker such as CK can be used to confirm the diagnosis, monitor

the progress of the patient in hospital and to gauge infarct size.

Creatine Kinase Muscle Brain Isoenzyme

The CK-MB isoenzyme has been considered as the gold standard for the diagnosis of AMI. It is the isoenzyme of CK specific for heart muscle. It is measured in serum or plasma by one of two methods: 1) CK-MB activity: this measures the total activity of the enzyme in serum/plasma by methods like electrophoresis, column chromatography, immunoinhibition or immunoprecipitation. The results are reported as IU/L. These methods are non-specific, measure only active enzymes, and have a low analytical sensitivity (5 IU/L); 2) CK-MB mass: this measures the protein mass in serum/plasma using specific antibodies against the M, B or MB subunits. The results are reported in ng/ml or µg/l. These assays are highly specific and have a high analytical sensitivity (0.3ng/ml) and can measure both active and inactive enzymes. The reference ranges using various methods have been reported to be 8-16 IU/L for CK-MB activity, and 5-10 ng/ml (µg/l) for CK-MB mass.[10]

Creatine Kinase-MB Activity versus Creatine Kinase-MB Mass

Different groups have reported that measurement of CK-MB using mass is better than that measuring CK-MB activity.[20] The recent guidelines for the redefinition of AMI recommend the use of CK-MB mass as opposed to CK-MB activity.[4] The problems inherent in measuring activity are:

1. The enzyme may become deactivated by experimental manipulations leading to a low value or underestimation. This is likely to happen in the low range of CK-MB activity such as in patients with small AMI or with minor myocardial damage. In an extreme situation, a false negative result could result in the missed diagnosis of AMI. Unlike CK-MB activity assays, CK-MB mass assays measure both active and inactive enzyme.
2. In haemolysed blood samples, interference from adenylate kinase, an enzyme released from red blood cells which catalyses the same reaction as CK-MB, could result in false positive results. This problem is irrelevant with mass assays because the antibodies are specific for the CK-MB and the presence of adenylate kinase has no effect on the assay.[17]
3. Creatine kinase-MB measurement using mass has better early sensitivity for the diagnosis of ST elevation AMI. It has also been reported to be more sensitive in detecting small injuries to the myocardium that occur in patients with non-ST elevation ACS.[21]
4. The presence of CK-BB, macro CK Type 1, or macro CK Type 2 in high concentrations could interfere with the result and lead to false elevation of CK-MB when activity measurement is used. These interferences have no such effects when mass assays are used because the antibodies are specific for CK-MB and do not cross-react with these compounds.[22]

Creatine Kinase-MB and ST Elevation Myocardial Infarction

Creatine kinase MB follows the same release kinetics as CK and has a sensitivity and specificity for the diagnosis of AMI of more than 90%. This sensitivity and specificity changes with the time of presentation after symptom onset. Measurement of CK-MB activity is most reliable in the 12-24 hours period after symptom onset.[23] A negative result earlier than 12 hours from the start of symptoms is too early to rule out AMI, and a negative result after 24 hours may be too late. Studies comparing the utilisation of these assays for the early diagnosis of AMI have shown that CK-MB mass reaches the cut-off point in serum several hours before CK-MB activity and have claimed its superiority within 4-8 hours after symptom onset.[21] The CK-MB mass assay has been shown to be sensitive for the diagnosis of AMI even in situations where the ECG is equivocal and can increase the number of diagnoses made within the time scale required for the administration of reperfusion treatment.[24]

Creatine Kinase-MB and Non-ST Elevation MI

Creatine kinase MB activity tends to be normal in patients with Non-ST elevation MI, whereas CK-MB mass is increased in a proportion of these patients. This observation has been substantiated by different groups.[25] A study by Seo *et al.* in 1993

compared CK-MB mass versus CK-MB activity and concluded that CK-MB mass is more sensitive when CK-MB concentration is in the low range.[26] This is important especially in cases of myocardial injury in Non-ST elevation MI, following complicated PCI, and in radiofrequency ablation of arrhythmias when the amount of CK-MB released can be small. Sensitive CK-MB mass assays can detect prolonged ischaemia in Non-ST elevation MI and have been used for risk stratification in these patients. One small study has shown a very high death rate (64%) during four years follow-up in patients who were admitted to the CCU with chest pain, positive CK-MB mass, and non-diagnostic ECG changes of ST elevation MI.[27] Several investigators have also studied the release of CK-MB mass following PCI.[28] They demonstrated that CK-MB mass is a sensitive indicator of myocardial injury following PCI. One study reported that 40% of patients showed evidence of myocardial damage following PCI using both CK-MB mass and cTnT.[29]

LIMITATIONS AND FUTURE DIRECTION OF CREATINE KINASE-MB ASSAYS

Creatine kinase MB measurement is not totally specific for MI and there are various causes for elevated CK-MB concentrations other than AMI.[17] Cardiac pathologies like congestive cardiac failure and arrhythmia lead to elevated CK-MB concentration. Severe skeletal muscle damage e.g. acute muscle trauma, skeletal muscle disorders like myositis, polymyositis, chronic inflammatory and degenerative muscle disorders can lead to CK-MB elevation. In these situations, the CK-MB to CK ratio or cardiac troponins can be used to differentiate cardiac and non-cardiac pathologies.[30] Measurement of CK-MB is a widely accepted assay that is both sensitive and relatively specific for the detection of AMI. However, CK-MB (activity or mass) is not sufficiently sensitive in the first 6 hours after symptom onset for the early diagnosis of AMI.[4]

CREATINE KINASE MUSCLE BRAIN ISOFORMS (SUBFORMS)

Creatine kinase MB isoforms are variants of the CK-MB isoenzyme, which result from post-synthetic modification of the M subunit. They were discovered by Weavers in 1972.[31] After ischaemic damage to the heart, the CK-MB isoenzyme is released from the damaged heart muscle into the blood. This isoenzyme is converted into other forms by the action of the plasma enzyme carboxypeptidase N (CPN) according to the following reaction: [32]

CK-MB2 (tissue form) ⇆ CPN (-Lysine) ⇆ CK-MB1 (plasma form)

In this reaction, the CPN enzyme removes one lysine amino acid from the M subunit of the released CK-MB2 to produce CK-MB1. CK-MB2 and CK-MB1 exist in equilibrium (1:1 ratio) in the serum of normal healthy people. Therefore, measurement of total CK-MB at any point in time is equivalent to 50% CK-MB2 and 50% CK-MB1.

Most assays that measure CK-MB use a relatively high upper limit of reference range (10ng/ml, 14 IU/L). Humans differ in their background level of CK-MB and some people may express normal concentrations as low as 1-2 IU/L (or ng/ml). Therefore, in the event of myocardial injury, it will require a several-fold increase in the marker before it exceeds the upper limit of the reference range. CK-MB, being a relatively large molecule, may take even longer to reach the circulation and become important diagnostically. When myocardial injury occurs, there is a sudden release and rise of the tissue isoforms, i.e. CK-MB2 compared to the plasma form CK-MB1, leading to a rise in the ratio of CK-MB2 to CK-MB1. By using CK-MB isoforms effectively each patient acts as his own control, and a release of only a small amount of the marker is required to raise the ratio much earlier to a significant level. The requirement for the diagnosis of AMI is two-fold: 1) increase of CK-MB2 > 2.6 IU/L and 2) increase of CK-MB2 to CK-MB1 ratio > 1.7.

CREATINE KINASE-MB ISOFORMS AND ACUTE MYOCARDIAL INFARCTION

Creatine kinase MB isoforms are reported to be released within 1 hour after symptom onset and peak at 4 hours. Evidence of AMI can be detected as early as 1-2 hours post-infarction, several hours before total CK-MB reaches diagnostic level.[33] These release kinetics make CK-MB isoforms potential markers for the early diagnosis of AMI. The sensitivity and specificity of CK-MB2 to CK-MB1 ratio for the diagnosis of AMI was reported to be 92% and 95% respectively within 6 hours of infarction.[34] During this time interval, total CK-MB

mass or activity would just be approaching the upper limit of the reference range. One study reported a sensitivity and specificity of 95.7% and 93.9% respectively with a high positive predictive value and a high negative predictive value within 6 hours of infarction. A total of 114 out of 118 patients with AMI were identified using CK-MB isoforms within 6 hours. The sensitivity and specificity of conventional CK-MB during this time interval was 48% and 94% respectively.[35] Seventeen patients who were discharged from the emergency department fulfilled the criteria for the diagnosis of AMI using CK-MB isoforms (missed diagnosis). The test was also positive in patients with hypothyroidism and rhabdomyolysis.[35] These findings were also substantiated by other studies.[36]

Some investigators however, have questioned the value of CK-MB isoforms for the early diagnosis of AMI. A study by Laurino *et al*. in 1996 showed no difference between CK-MB isoforms and conventional CK-MB isoenzyme at 6 hours after symptom onset.[37] Another study by Bhayana *et al.,* using comparison between CK-MB mass, CK-MM3 to CK-MM1 ratio, and CK-MB2 to CK-MB1 ratio found no significant advantage of isoforms over CK-MB mass for the diagnosis of AMI within 6 hours.[38]

LIMITATIONS OF CREATINE KINASE-MB ISOFORMS ASSAYS

There are still unresolved issues surrounding the use of CK-MB isoforms for the early diagnosis of MI. There is still some concern regarding the stability of these isoforms in serum/plasma after their release from damaged tissues.[39] There are also some reports of possible variations in the activity of the CPN enzyme among humans.[40] This may have a profound effect on the use of the isoforms as markers for the early diagnosis of AMI and could lead to false positive or false negative results.[39] The other drawback is that the use of isoforms does not obviate the need to analyse total CK and the relative index of CK-MB to total CK to exclude false positive results.[41]

Myoglobin

Myoglobin is a small heme protein (17 K Da) that functions in oxygen binding and transport. It stores oxygen in red muscles (skeletal and cardiac) and, under conditions of severe oxygen deprivation, it releases the oxygen to be used by muscle mitochondria for synthesis of adenosine triphosphate (ATP). The myoglobin content of heart muscle is reported to be 2.5mg/g wet weight of tissue and the skeletal muscle content is 4.0mg/g wet weight of tissue.[42] Myoglobin constitutes 2% of the total cytosolic protein content of cardiac muscle. The reference range of serum myoglobin is about 20-80ng/ml. Males have higher levels than females because they have bigger body size and muscle bulk.

MYOGLOBIN AND ACUTE MYOCARDIAL INFARCTION

Myoglobin is one of the best available early markers of AMI within 3 hours after symptom onset. The relationship between AMI and high myoglobin levels was first reported in 1975.[43] Myoglobin starts to increase in blood within 2 hours after symptom onset of AMI, peaks at 6-9 hours, and returns to normal within 24 hours. In a study by Bhayana in 1994, myoglobin was found to be superior to CK-MB mass and TnT for ruling out AMI within the period of 3-6 hours after symptom onset.[44] This early release feature of myoglobin is attributed to its small size and localisation within the cytosol of the cell. Several investigators have confirmed a significant role for myoglobin in the early diagnosis of AMI, the most promising role being in the early exclusion of AMI in patients presenting within 6 hours after symptoms onset.[45] Within this period, the overall diagnostic sensitivity and specificity ranged from 77-97% and 90-97.9% respectively. The variation in sensitivity depends mostly on the time of presentation after symptom onset and drops considerably with very early (< 2 hours) or late presentation (> 15 hours) to the hospital.[11] A consistently negative result within this time interval has such a high negative predictive value for ruling out AMI that confident decisions regarding patients' management can be based on it. However, a positive result should be used with caution, as there are many situations that could give rise to myoglobin elevation in the absence of AMI.[46]

LIMITATIONS OF MYOGLOBIN MEASUREMENT

Myoglobin is a non-specific marker protein for myocardial injury. Serum myoglobin is raised in

skeletal muscle damage including intramuscular injection, exhaustive exercise, muscle trauma, direct current shock cardioversion, and also in patients and carriers of genetic muscle disease. Severe renal disease leads to failure of clearance of myoglobin from the circulation. The concentration tends to rise and the circulation time is prolonged in these patients. Although these factors interfere with the specificity of the test, in clinical practice most of them could be ruled out by careful attention to history taking and simple blood tests. The specificity is increased when myoglobin measurement is combined with other diagnostic methods like ECG or more specific cardiac markers like CK-MB, cTnT, or cTnI.[47]

MYOGLOBIN AND THE DETECTION OF REPERFUSION

Ideally, all AMI patients should be treated with intravenous thrombolytic treatment or PCI. The success of thrombolytic treatment in establishing reperfusion is reported to be 50-80% depending on the thrombolytic agent.[48] Establishment of reperfusion after the initiation of thrombolytic treatment is important to clinicians in terms of treatment and prognostic implications. Coronary angiography is the most definitive method to assess the success of thrombolytic treatment, but this procedure is invasive, carries morbidity and mortality risks, requires a catheterisation laboratory team, and is not widely available. Biochemical markers like myoglobin (among others) offer an alternative non-invasive, safe and potentially sensitive method for the detection of reperfusion. This can be done by monitoring the changes in serum concentration immediately before and 60 or 90 minutes after initiation of thrombolysis. Patients who successfully reperfuse their occluded artery show a higher and early concentration peak of the biochemical marker compared to those who fail to reperfuse.[49] Patients with uncomplicated MI who reperfuse achieve a peak value of myoglobin at 1 hour, whereas those who fail to reperfuse reach peak value about 5 hours later.[50] A two-fold increase in myoglobin concentrations within 60 minutes compared to the base line pre-thrombolysis value is associated with 95% predictive accuracy for the detection of reperfusion.[51] The existence of "no-reflow" is an important indicator of impaired reperfusion and functional recovery. Thus a patent artery does not invariably indicate tissue reperfusion. Accurate markers of tissue reperfusion are required. The combination of serial 12-lead ECG, clinical features, and serial cardiac markers testing offer a practical alternative to coronary angiography for assessment of reperfusion status.

Heart-type (H-FABP) Fatty Acid-Binding Protein

Heart-type fatty acid binding protein (H-FABP) is a small (15 Kda) soluble non-enzyme protein. It is composed of 132 amino acids. It is one of the most abundant proteins in the heart and comprises 5-15% of the total cytosolic protein pool in the aqueous cytoplasm. It was introduced by Glatz *et al*. in 1988 as a potential novel biochemical marker for the early diagnosis of AMI;[52] this was soon confirmed in other studies.[53-54] Under normal conditions, H-FABP is not present in plasma or interstitial fluid, but is released into the blood upon cardiac cellular injury. The cytoplasmic to vascular concentration of H-FABP is of the order of 200,000:1.[55] The plasma or serum concentration of H-FABP under normal conditions is < 5µg/L. This makes the plasma estimation of H-FABP a suitable indicator for the early detection and quantification of myocardial tissue injury.

H-FABP AND ACUTE MYOCARDIAL INFARCTION

Heart-type FABP is released into plasma within 2 hours after symptom onset and is reported to peak at about 4-6 hours and to return to normal base line level in 20 hours.[55] Within the period of 30-210 minutes after symptom onset, H-FABP has > 80% sensitivity for the diagnosis of AMI.[56] Within the interval of 0-6 hours after symptom onset, the other cardiac markers such as CK, CK-MB (mass or activity), I cTnI and cTnT will only be starting to accumulate in the plasma, and their sensitivity has been reported to be around 64%.[57] A rise in serum and urine H-FABP concentration above reference values is seen in patients presenting with AMI as early as 1.5 hours after symptom onset. Serial measurements of H-FABP in the first 24 hours after onset of symptoms may be potentially useful for: the diagnosis of AMI; to identify patients who need early reperfusion treatment; to identify patients who reperfuse their infarct related artery; to detect

re-infarction if it occurs early and for estimation of infarct size.[58] However, some of the more recent studies have questioned the value of these early markers (H-FABP and myoglobin) when compared with specific markers like cTnI.[59-60]

LIMITATIONS AND FUTURE DIRECTIONS OF H-FABP MEASUREMENTS

Heart-type FABP exists in high concentrations in the heart only. However, this protein is not totally cardiac specific and occurs in other tissues although in much lower concentrations.[57] It is present in skeletal muscles in concentrations varying between 0.05-0.2 mg/g wet weight of tissue, depending on the muscle fibre type studied.[61] It has also been reported in very low concentrations in tissues like the kidney, aorta, testes, mammary glands, placenta, brain, adrenal glands, adipose tissue, and stomach.[57]

Heart-type FABP is secreted by the kidney and circulates for a longer time (> 25 hours) after AMI in the presence of renal failure. Gorski *et al.* reported that H-FABP and myoglobin concentrations were both significantly elevated in patients with renal failure. The concentrations of these markers were not affected by dialysis.[62] We have also shown that the efficiency of H-FABP for the diagnosis of AMI is severely limited in patients with renal failure.[63] Skeletal muscle damage during the course of AMI, e.g. intramuscular injections, electric cardioversion and traumatic cardiopulmonary resuscitation, may result in the leakage of H-FABP and this could interfere with the results of the assays.[64] Diagnosis of AMI in these groups of patients using H-FABP alone can be difficult. H-FABP is increased in the plasma of healthy volunteers after strenuous exercise.[65] Surgery (both cardiac and non-cardiac) causes elevation of H-FABP concentration.

H-FABP AND MYOGLOBIN

Myoglobin and H-FABP share many key features: 1) low molecular weight proteins (17 and 15Kda, respectively); 2) abundant concentrations in the cytosol of myocardial cells; 3) substrate for mitochondrial oxidation (oxygen and fatty acids, respectively and 4) both are released within 2 hours after symptom onset, peak early (6 hours) and return to normal baseline concentration within 24 hours. Both proteins are present in the heart and skeletal muscle in different concentrations. The concentration of myoglobin in heart and skeletal muscle is 2.5 and 4.0mg/g wet weight of tissue, respectively. The H-FABP concentration in heart and skeletal muscle is 0.5 and 0.05-0.2 mg/g wet weight of tissue, respectively. The myoglobin content of skeletal muscle is twice that of the heart. The H-FABP content of skeletal muscle is only 10-50% of that of the heart. The normal plasma concentration of H-FABP (< 5µg/L) is 10 to 15-fold lower than that of myoglobin (30-80µg/L). H-FABP is therefore more cardio-specific than myoglobin. The normal ratio of myoglobin: H-FABP in the myocardium is about 1:5, whereas the ratio in skeletal muscle is in the order of 21:70 (depending on muscle type).[66] The main disadvantage of myoglobin and H-FABP as early markers of myocardial injury is their lack of total specificity due to their presence in skeletal muscle. Because both proteins are released into plasma after injury at about the same time and in a ratio similar to the protein's concentration in the tissue of origin, the measurement of the myoglobin: H-FABP ratio could be a useful index for discrimination between cardiac and skeletal muscle damage. A myoglobin: H-FABP ratio that is around 1:5 is considered to be specific for the heart and a ratio that is between 21:70 is more specific to skeletal muscle damage.[64] The ratio has been reported by some investigators to increase the diagnostic specificity for the diagnosis of AMI more than relying on either marker alone. However, the use of this ratio should not be a rigid criterion as overlaps do occur. Some investigators did not support additional value over the measurement of H-FABP alone.[64,67]

Cardiac Troponins

The troponin complex is found on the thin filament (actin) of all types of striated muscle (fast, slow, and cardiac). Its function is to regulate calcium dependent contraction of muscles. There are three types of troponins: TnT, TnI and TnC. They are designated with a letter that refers to the function of the troponin protein; TnC binds calcium; TnI inhibits the action of the enzyme actomyosin adenosine triphosphatase; TnT binds to tropomyosin.[68] They are called isoforms and have a pre-fix to indicate the muscle type they are in e.g. cTnT, sTnT, fTnT stand for cardiac muscle, slow twitch skeletal muscle, and fast twitch skeletal muscle TnT respectively. Cardiac TnT has more tissue distribution and more free cytoplasmic

concentration and is released as a complex with the other cardiac troponin T-I-C. Cardiac TnI is released more in the binary form (troponin I-C complex).[69] Each troponin protein within these muscles has a different molecular weight, different amino acids, and an amino acid sequence unique to that muscle type. The different isoforms of TnT and TnI share between 40-55% of amino acid sequence homology.[70]

CARDIAC TROPONIN T AND ACUTE MYOCARDIAL INFARCTION

Cardiac-TnT (34 KDa) was first introduced in 1989 as a marker for AMI.[71] The upper limit for cTnT has been reported as < 0.1µg/L, but concentrations between 0.03-0.1µg/L may also have significance as markers of an adverse outcome.[72] Cardiac-TnT appears in the serum within 12 hours after symptom onset in patients with AMI. It shows similar release kinetics to CK-MB and cTnI, and thus does not provide an earlier detection for AMI than CK-MB or cTnI within the first 6 hours after symptom onset.[73] Once in the circulation, it persists for a long time (2-3 weeks) after symptom onset. The half-life of cTnT in circulation is 120 minutes and this long diagnostic window is thought to be due to the continuous release of the marker from myocardial cells after necrosis, and not due to slow clearance from the circulation.[74] The clinical sensitivity of cTnT for the diagnosis of AMI approaches 100% at about 12 hours after symptom onset and remains elevated at 100% for at least 4 days.[74]

UNSTABLE ANGINA AND INCREASED CARDIAC TROPONIN T (NON-ST ELEVATION MI)

Unstable angina/Non-ST elevation MI carries significant morbidity and mortality risks and early detection and treatment are essential to minimise complications.[75] Cardiac-TnT has been shown to be elevated in patients with Non-ST elevation MI and the magnitude of elevation can be used for diagnostic purposes, risk stratification, and for selection of appropriate patient groups for treatment. Elevated cTnT in patients with Non-ST elevation MI is associated with poor prognosis.[76] The increase correlates well with the severity of coronary artery lesions determined by angiography.[77] In one important study, the prognostic value of cTnT was assessed in 967 patients with unstable angina. It was found that the group that had elevated concentrations of cTnT had an increased risk of cardiac events and the higher the cTnT the more frequent the complications. Patients were followed-up for 6 months for cardiac complications. The risk of further AMI and death was 4.3% in patients with cTnT less than 0.06µg/L and 16.1% for those with cTnT equal or greater than 0.18µg/l.[78] In another study by Stubbs *et al.*, 62 Non-ST elevation MI patients were followed-up for about three years after their admission with cTnT concentration greater or equal to 0.2 ng/ml. The incidence of complications in this group was very high: cardiac death (12 patients), coronary revascularisation (22 patients), death and non-fatal AMI (18 patients).[79]

Cardiac-TnT measurement can help select the appropriate patients for treatment with antithrombotics. Treatment of Non-ST elevation MI patients with thrombolytic therapy had not been found to be useful.[80] However, treatment of Non-ST elevation MI with antiplatelet and antithrombotic drugs like aspirin, clopidogrel, low molecular weight heparin (LMWH) and glycoprotein IIb/IIIa (GP IIb/IIIa) receptor antagonist was associated with 30% reduction in the incidence of mortality and AMI.[81] Those patients with cTnT < 0.1 ng/ml fared equally well whether they were treated by LMWH, deltaparin or a placebo (4.7 versus 5.7% x 40 day mortality respectively). However, patients with cTnT greater than or equal to 0.1ng/ml had reduced AMI and mortality if they received deltaparin rather than a placebo (7.4 versus 14.2% respectively).[82-]

SPECIFICITY OF CARDIAC TROPONIN T ASSAYS

Elevated cTnT concentration has been reported in a significant numbers of patients with chronic renal failure. These levels do not seem to be affected by haemodialysis, with elevations persisting after treatment.[83] In chronic kidney disease patients on haemodialysis without acute coronary syndrome, cTnI was reported to have better sensitivity and specificity for the diagnosis of AMI compared with cTnT, but positive cTnT was associated with increased all cause mortality during follow up.[84-85] Vigorous exercise, e.g. marathon runners; rhabdomyolysis; inflammatory muscle disease, e.g. polymyositis and dermatomyositis, and degenerative muscle disease, e.g. Duchenne/Becker

muscular dystrophy, have been reported to show elevated concentrations of cTnT.[86] High cTnT and cTnI concentrations have been reported in critically ill patients who had not been diagnosed with comorbid AMI.[87] Blunt trauma to the chest, closed heart massage, external defibrillation is also reported to result in elevation of cTnT.[88] Elevations have also been reported in cases of myocarditis and drug induced cardiac toxicity.[89,90] Elevated troponin levels (I and T) occur in some patients with acute pulmonary embolism and this elevation has been associated with a high risk of short-term death and an adverse outcome events.[91] The advantage of the cTnT immunoassay is that currently it is marketed by one source only hence there are well-established standards in terms of reference range, detection limits, and clinical cut-off concentrations. There is however, a slightly higher rate of positive results with cTnT assays in some patients with chronic renal failure and acute or chronic muscle disease.[86,92]

CARDIAC TROPONIN I AND ACUTE MYOCARDIAL INFARCTION

Cardiac-TnI (24 KDa) is reported to have a unique segment containing 31 amino acids that makes it different to either sTnI or fTnI (19 KDa). During foetal development both sTnI and cTnI are expressed in the myocardium; however, at birth, the cTnI remains as the only isoform present in the human myocardium.[93] Cardiac-TnI has not been shown to be expressed in any type of skeletal muscle during either development or disease stimuli.[94] This makes cTnI 100% specific for the myocardial tissue and an excellent marker for the detection of myocardial injury in serum. Cardiac-TnI was first reported as a biochemical marker of myocardial injury in 1992 and has since been shown to be a very sensitive and specific marker for the diagnosis of AMI. It has similar release kinetics to CK-MB and to cTnT and does not provide an earlier detection for AMI within the first 6 hours after symptom onset.[95] Cardiac-TnI peaks between 12-36 hours after onset of AMI and remains elevated for 3-7 days after AMI. The half-life of cTnI is < 2 hours and the prolonged diagnostic window is due to the continuous release of this marker from the myofibril.

CARDIAC TROPONIN I AND NON-ST ELEVATION MI

Cardiac-TnI is cardiac specific and its concentration in normal and disease free populations is undetectable or very low. This makes it a suitable marker for the detection of myocardial injury in patients with Non-ST elevation MI as well as other situations where myocardial injury is expected, but the amount released could be small, e.g. following PCI.[96] Many studies have also shown cTnI to have prognostic value in patients with non-ST elevation MI similar to that of cTnT. Those patients showing higher levels at admission have more complications increased mortality and AMI on subsequent follow-up.[97] The prognostic risk in these patients is significantly altered if early intervention (pharmacological and invasive) is undertaken in these patients compared to conservative treatment.

SPECIFICITY OF CARDIAC TROPONIN I ASSAYS

Cardiac-TnI was not found in patients who underwent uncomplicated angioplasty.[98] In patients with chest trauma, cocaine associated chest pain, and hypothyroidism where CK and CK-MB were elevated, cTnI was able to distinguish true myocardial injury from cases with false elevation. In marathon runners, more than 80% of samples were positive for CK-MB and in all of these samples cTnI was negative. Cardiac-TnI was found to be significantly elevated (43%) in patients with bacteraemia.[99] The main problem with cTnI is that there are several commercial assays available, and each assay differs with respect to reference range, detection limit, and medical decision cut-off limits. Thus, there is a lack of standardisation of methodology for cTnI assays. However, cTnI has been reported to show slightly better specificity in situations where there is severe skeletal muscle injury and renal failure.[84,85]

SPECIFICITY OF CARDIAC TROPONINS FOLLOWING SURGERY

Cardiac-TnI was not elevated in patients who underwent non-cardiac surgery, but was raised in patients undergoing CABG due to surgical injury of the myocardium. Cardiac-TnI or cTnT may be the preferred markers of choice to detect myocardial injury in patients who undergo non-cardiac surgery. In the situation of non-cardiac surgery, cTnI (or cTnT), being specific to the myocardium, will also

help to distinguish elevation of CK-MB due to skeletal muscle damage alone from elevation due to myocardial injury.[100]

Conclusion

The markers that are well suited for the early diagnosis of AMI within the time interval 0-6 hours after symptom onset are myoglobin, H-FABP and CK-MB isoforms. Although all have been shown to be excellent sensitive early markers, there are still significant issues concerning its specificity. H-FABP is more cardio-specific than myoglobin and the use of H-FABP as a marker for the early diagnosis of AMI seems preferable. CK-MB mass measurement is suitable in the 6-24 hours interval; CK-MB based on activity measurement is more sensitive in the 12-24 hours interval, and the other cardiac markers like total CK, cTnT, and cTnI are most reliable after 12 hours from symptom onset. The prolonged diagnostic window of cardiac troponins of several days that is highly sensitive and specific obviates the needs for less specific markers with long diagnostic window like aspertate aminotransferase (ALT) and CK. Decision-making regarding triage and treatment of patients should not be based on a single measurement of cardiac markers alone because of the time delay required for the marker to exceed the upper limit of reference range. Based on the recent recommendations by the ESC/ACC, cTnI and cTnT are the best markers for the confirmation of AMI. CK-MB (preferably mass) is the second best marker in the absence of troponins assays. Patients who present with acute chest pain suspicious of ACS are best managed within specifically designated units that have rapid access to specific equipment (ECG, echocardiogram) and facilities (point of care instruments to measure cardiac markers), and with appropriate staffing. Acute chest pain units attached to emergency departments can accommodate patients for 12 hours, so that appropriate investigations can be carried out quickly after presentation in order to identify patients with evolving infarction who are at increased risk.

A serial combination testing of a sensitive early marker (e.g. H-FABP, myoglobin or CK-MB isoforms) and one of the cardiac specific troponins (cTnT or cTnI) offers the best approach. Two serial testings within a minimum of 12 hours (e.g. at 0 hour, 4-6 hours, or at 12 hours) after symptom onset provide reliable sensitivity and specificity for detecting ischaemia and evolving infarction within the time interval required for the implementation of reperfusion therapy. This 12 hour strategy also identifies patients at low-risk for acute ischaemic events.

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