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An overview of the significance of troponin in acute myocardial infarction

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~ ABSTRACT ~

Biomarkers are helpful tests for making a diagnosis. They are usually the golden standard for their respective diseases. Cardiology, especially, uses biomarkers extensively. Because a great margin of cardiologic emergencies present with a risk of mortality; swift diagnosis and treatment is essential. Biomarkers are helpful in the manner that they shorten diagnosis time. Acute Myocardial Infarction (AMI) is one of the most frequent cardiological emergencies. When it is taken into account that AMI presents with vital risk and that the outcome is dependent on swift diagnosis and treatment speed; it is obvious that it is of utmost importance to have a biomarker that defines the diagnosis with certainty. Cardiac troponin harbors these qualities and is accepted as the fundamental factor for diagnosis and prognosis. This review summarizes the start of biomarker research for AMI until the discovery of troponin, emphasizes troponin's usage today and provides possible ideas for future enhancement.

Key words: myocardial infarction. cardiac biomarker, troponin, cTnI, cTnT

Introduction

Generally speaking, a biomarker is anything that can be used as an indicator of a particular disease state, some physiological process, or the response to a therapeutic intervention of an organism [1]. Biomarkers are often the golden standard for their respective diseases [2]. It is faster, easier and usually cheaper to have a specific biochemical indication and/or proof of an illness than to have an extensive amount of clinical and/or biochemical factors/findings that could have different interpretations by physicians based on situational differences. This also establishes a requirement for a good biomarker; that it should help a diagnosis or make a diagnosis possible in the first place [3]. Any physician will accept that seeing the result of a specific biomarker for any suspected disease will set their mind at ease even if they are relatively sure of their diagnosis.

When cardiology is taken into account, this situation becomes even more apparent; cardiologic emergencies often present with life or death situations. The ER physicians have limited time to diagnose these emergencies; and after the diagnosis; even less (usually immediate) time to start treatment [4].

Acute Myocardial Infarction (AMI) is a fast emerging, life threatening emergency for cardiology. The need for swift and reliable diagnosis is apparent for AMI when this setting is taken into account.

These reasons identify the search and extensive use of biomarkers in cardiologic emergencies. The purpose of this review is to understand the historic development of AMI biomarkers leading to today's golden standard troponin, its usage and to ask questions about the future of troponin for AMI.

Acute Myocardial Infarction

Simply put, AMI is the blockage of the coronary blood supply leading to insufficient oxygen flow to any area of the heart. It being "acute" indicates that the blockage occurred suddenly. Cardiac muscle regeneration is very low and if left untreated, AMI will result in cardiac muscle necrosis and in turn; increased mortality and morbidity. [5]

The short life span of cardiac muscle in the absence of oxygen and the potential loss of cardiac function dictates the need for swift diagnosis. These reasons are the basis for biomarker research

for AMI. The affect of biomarkers on AMI diagnosis and treatment will be discussed further in the article.

The ideal biomarker of MI should [6]:

- Provide early detection of myocardial injury
- Provide rapid, sensitive and specific diagnosis for an AMI
- Serve as a risk stratification tool in acute coronary syndromes (ACS)
- Assess the success of reperfusion after thrombolytic therapy
- Detect reocclusion and reinfarction
- Determine the timing of an infarction and infarct size
- Detect procedural-related perioperative MI during cardiac or noncardiac surgery

However, there has not been such a biomarker for the time being.

First Footsteps

The first usage of biochemical markers in acute myocardial infarction (AMI) is dated back to 1955, when Karmen et al. reported increased aspartate aminotransferase (AST) levels arising from necrotic heart muscle tissues [7]. Today, AST is used as a secondary to Alanine aminotransferase (ALT) activity for liver function tests. After AST, lactate dehydrogenase (LDH) activity was discovered. [8] LDH is an enzyme found in nearly all body tissues. Any type of tissue breakdown releases LDH. Today it is used primarily as a minor guideline for different diseases (Cancer, Acute Pancreatitis) and their therapies; rather than a marker for a specific disease. Later, creatine kinase (CK) activity in serum was established. CK had a very distinct increase and decrease after AMI and had better specificity for muscle injury. CK is still used as an early indicator of muscle injury because the rise of CK after muscle trauma is almost instant. But none of these markers were cardio-specific [9-10].

The Search for Specificity

The search for a cardiospecific biomarker resulted in the age of CK-MB. CK-MB was an isoenzyme of CK; but had the advantage of corresponding to nearly 22% of CK in cardiac muscle, compared to just 2% in skeletal muscle [10]. The use of CK-MB revolutionized the approach to AMI and was the basis for establishing new diagnostic guidelines.

In 1979 World Health Organization (WHO) recognized the role of CK, CK-MB, LD, and AST activities and included them in its classical AMI criteria [11]:

1. Clinical history of ischemic type chest pain lasting for more than 20 minutes
2. Changes in serial ECG tracings
3. Rise and fall of serum cardiac biomarkers

These criteria are -although a number of changes have been made- still used to this day. Until 2000, two out of three were accepted as a probable diagnosis while three out of three was defined as definite AMI. The modern version (defined in 2000; refined in 2007 and 2012) states the biomarker criteria as a must and adds "imaging evidence of new loss of cardiac muscle," "ECG evidence of new left bundle branch block (LBBB)," "identification of an intracoronary thrombus" as other supporting criteria [12-13]. So a diagnosis of AMI without invasive procedures or postmortem studies cannot be made unless cardiac biomarkers are not elevated [13].

In the 80's, CK-MB was accepted as the first choice test for AMI diagnosis but it was still apparent that the testing technique and speed had to be improved and false negatives/ positives owing to the unspecific (increases originating from muscular stress or disease) nature of CK-MB should be addressed. It is such that CK-MB specificity is lowered drastically by skeletal muscle damage; although CK-MB is in very low amounts in skeletal muscle, the sheer size of skeletal muscle mass can create enough CK-MB that will result in false positives when the patient history includes situations like large traumas, heavy exercise and muscular atrophy [10]. This sparked the re-emergence of large studies trying to find the marker that was specific only to the cardiac muscle, "the marker that would rule them all".

The Troponin Era Begins

After extensive studies, cardiac troponin I (cTnI) and T (cTnT) were discovered in close succession [14-15]. While troponin exists in skeletal (sTnI and sTnT) and cardiac muscle, cardiac specific versions (cTnI and cTnT) are used to identify AMI. cTn provides the strength to be utilized in the majority of clinical areas mentioned for an ideal biomarker of MI in Part 2.

The first commercial cTnI assay for the Stratus I analyzer (Dade Behring) appeared in 1996 [16]. The relationship between troponin and AMI is evaluated by the characteristic rise and fall in its serum values. This association can be briefly explained as:

"following cardiac cell necrosis, troponin elevation starts within 2-3 hours, peaks in 16-24 hours, and persists for 1-2 weeks" [17].

What is Troponin?

Troponin (Tn) is a complex of contractile regulatory proteins that are found in cardiac and skeletal muscle but not in smooth muscle (Figure 1). Tn complex consists of 3 sub-units, namely troponin C (the calcium binding component), troponin I (the inhibitory component) and troponin T (the tropomyosin binding component). Troponin T binds to tropomyosin which regulates the (in)activation of actin. In the absence of calcium, tropomyosin stays inside the grooves of actin and inhibits contraction. When calcium enters the cell after neural activation, troponin C binds the calcium that enters the cell and changes the conformation of the molecule to enable contraction [18]. The homology between cardiac and skeletal troponin is 56.6% for cTnT and sTnT, and 40% for cTnI and sTnI [19]. This homology is critical for analytic systems, and different measures are taken to distinguish cardiac troponins from skeletal troponins.
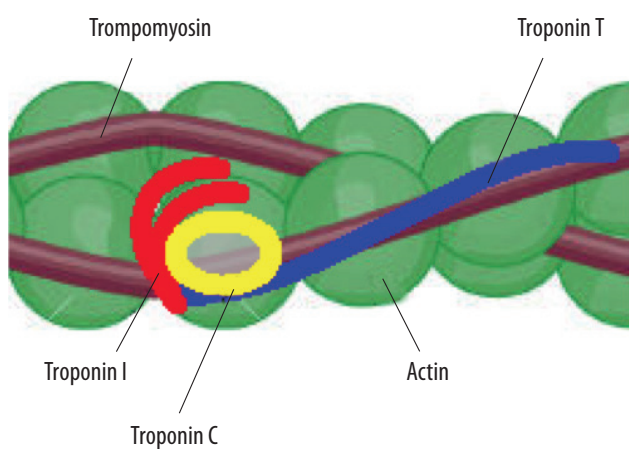

Figure 1. Schematic figure of troponin in human

After myocardial injury develops, multiple forms of troponins are elaborated both in tissue and in blood such as troponins T-I-C complex, I-C complex and free I. Various modifications such as oxidation, reduction, phosphorylation and dephosphorylation and both C- and N-terminal degradation can occur in these molecules [16]. Therefore cTn immunoassays should contain antibodies, which recognize epitopes in the stable region of cTnI to permit an equimolar response with various cTnI forms circulating in the blood after cardiac injury.

Analytical Methods for Measuring Cardiac Troponin

Cardiac troponin T and I are measured with high sensitivity by monoclonal antibody-based immunoassay methods in which assay times range from 5 to 30 minutes [20-21].

cTnT method is under patent, and due to regulations only Roche Diagnostics distribute cTnT. On the other hand, cTnI can be used without patent conflicts and is developed by many different immunoassay manufacturers [21]. However these multiple cTnI methods use different antibodies in their respective assays which recognize different epitopes of cTnI thus making the standardization of various cTnI assays elusive.

Although there was a big debate on which marker was 'the better troponin'; tests showed that both cTnT and cTnI showed similar diagnostic ability in detection of myocardial damage [22]. In some studies, cTnT was shown in the skeletal tissue of patients with chronic muscle diseases and injuries [23-24], which points to a lesser specificity for cTnT, thus the debate is continued.

False Positive Elevation in Analytical Methods

Troponin elevation is nearly always specific to cardiac injury; this is what makes the test a good one. However, in analytical systems mistakes happen due to various reasons. For -specifically- troponin quantification, some of the most frequent erroneous results are reported by the US-FDA to emerge from [25]:

- Fibrin clots in serum as a result of incompletely clotted specimen, in patients with coagulopathy or on anticoagulant therapy [26] [27]
- Endogeneous antibodies such as rheumatoid factor, heterophilic antibodies, human anti-animal antibodies, injection of antibodies for imaging/treatment procedures [28-29].
- Interference from other endogenous components in the blood such as bilirubin and hemoglobin [30].
- Immunotherapies, vaccinations or blood transfusions [31] and some form of immunocomplex formation [32].
- Any microparticles in specimen that interfere with the testing method at hand.
- High concentration of alkaline phosphatase [33].
- Analyzer malfunction [34].

To solve some of the specimen-related errors, ultra-centrifugation has been proposed with noted

success in reducing false positives [27]. Other methods for better testing are: dilution, use of heterophilic blocking tubes, immunoglobulin inhibiting agents, precipitation with polyethylene glycol [31].

Troponin Today

Troponin is accepted as the best indicator for cardiac injury. In 2000 a joint committee of the European Society of Cardiology and the American College of Cardiology (ESC/ACC) issued new criteria that stated elevations in biomarkers were fundamental to the diagnosis of acute myocardial infarction [12]; because the suffered AMI may be atypical or electrocardiogram changes may be absent or nonspecific. Any indicator beside troponin can be a false indicator for diagnosis or prognosis. For example normal or nonspecific initial ECG's for patients with AMI do not indicate that the patient will have a good prognosis. Quite adversely, patients who initiate with normal ECG's and are later diagnosed with AMI usually have a worse prognosis than patients who have ischemic symptoms on their ECG [35]. This means that today cardiac troponin is the most important part of an AMI diagnosis and best indicator for prognosis [13].

Although troponin test inherits very good specificity and sensitivity values, it should only be used in assistance to clinical decision making. Due to economical reasoning and possible differential diagnoses, the decision for testing should be made after there is reasonable evidence of acute myocardial injury. Elevated cTn values outside of ACS are not uncommon and reflect cardiomyocyte necrosis from a wide selection of cardiac, pulmonary and systemic diseases. [36]

Troponin Predicts Prognosis

During some trials, troponin was found elevated in one-third of patients whom AMI was ruled out using WHO criteria [37]. These cases were believed to be minor cardiac injury. When these patients were compared to patients who had low troponin levels, it was seen that prognosis for troponin elevated patients were worse. Additionally, it was found that In ST-segment elevation MI, a troponin T ≥0.1 μg/L on admission indicates poorer prognosis despite early reperfusion [38]. This solely shows the ability of troponin as an independent indicator of lost cardiac muscle and prognosis. Findings like these changed the management of acute coronary syndrome (ACS) patients to account for their troponin levels. [39]

Troponin, that is "better" than Troponin?

Biochemical markers come with a price. When the value for a marker is near the cut-off value of a diagnosis, but doesn't quite -cut- it; what does the clinician do? Retests are good to some measure; but if the test itself could get better at testing; it would do wonders for these grey areas in medicine. In case of AMI, ruling it in or out. This is the reason for research and development of high sensitivity cardiac troponin assays (hs-cTn). The term 'high sensitivity' refers to the assay's characteristics but not to a difference in the form of cTn being measured.

When hs-cTnT and cTnT were compared in a study by Ru-Yi Xu et al, introduction of the hs-cTnT assay with lower cut-off levels for diagnosing AMI was found helpful to clinicians in patients with acute chest pain [40]. hs-cTnT is not only better sensitivity/specificity-wise, it also has a faster result time than the standard cTnT assay. The gain in sensitivity and speed may be the difference between life and death for patients with a short duration from symptom onset to admission [40]. In addition to the cardiologic aspects of hs-cTn assays, they can be used to monitor myocardial injury due to ischemic strokes. Myocardial injury is detectable in more than half of the patients with acute ischemic stroke by using hs-cTnT according to findings of a recent study that involved about a thousand patients [41].

Concentrations of hs-cTn are expressed in nanograms per liter compared to usual units of microgram per liter. It is to bear in mind that with increased clinical sensitivity with the ability to detect smaller myocardial injuries due to various pathological etiologies, clinical specificity of the test decreases. Detecting Tn in the serum of a healthy individual has a low probability. However as more sensitive assays are being developed, Tn can be eventually detected in the sera of healthy people.

Conclusion

Troponin is undoubtedly the best test for cardiac necrosis; it has the best sensitivity and specificity values and helps prediction of prognosis from day one. However, it does not indicate the mechanism of damage to the tissue. The next step for troponin (or the next golden standard marker) could be furthering the specificity of the biomarker and/or gaining the ability to understand the mechanism of damage. As we have expressed quite often, time is essential for AMI diagnosis and treatment.

Although cardiology specialists are often on-call, AMI patients are usually evaluated by ER doctors who are not specialists of cardiology. Even the short time that it takes the specialist to get up to speed on the case can translate to lost cardiac muscle and

function. So, markers that could reveal the location of damage on the heart and/or a marker that could establish cut-off points for the decision for invasive or medical treatment could start a new era of cardiologic care for AMI cases.

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