

**ACUTE MYOCARDIAL INFARCTION: DEFINITION, DIAGNOSIS, AND THE  
EVOLUTION OF CARDIAC MARKERS**

**LEARNING OBJECTIVES:**

1. Define MI and the challenges in MI diagnosis
2. Define the current recommendation criteria for diagnosis of MI
3. Summarize the history and evolution of cardiac markers
4. Recognize the importance of troponin as a cardiac marker
5. Recall the historical development of troponin assays
6. Identify the advantages and disadvantages of high sensitivity troponin assay as a cardiac marker

**ABSTRACT:**

In the United States, heart disease is the leading cause of death and accounts for 1 in every 4 deaths each year. Coronary artery disease (CAD) is the most common type of heart disease and is the principle source for deaths due to heart disease. CAD refers to a group of diseases including stable angina, unstable angina, and myocardial infarction (MI). An estimated 735,000 individuals suffer an MI annually, with the majority (roughly 525,000) being first time heart attacks. The current diagnosis of MI is based on the rise and fall of cardiac markers, preferably troponin, with at least one measurement being above the 99th percentile Upper Reference Limit (URL), with strong clinical evidence. The advancement in cardiac troponin assays has provided an opportunity for clinicians to more quickly identify acute coronary disorders and to determine the best treatment protocol for patients. The fifth generation assays, known as high sensitivity cardiac troponin (hs-cTn) assays, were FDA approved in the US only in 2017. As we take a closer look at the high sensitivity troponin assay, we need to take into consideration issues related to this assay in all steps of the testing process (pre-analytical, analytical, and post-analytical). In this article, we define MI and the challenges that come with diagnosing MI, and the history and evolution of cardiac markers over the years is summarized. The future of MI and cardiac disease identification is described by identifying the advantages and disadvantages of high sensitivity troponin assays.

**ABBREVIATIONS:**

ACC - American College of Cardiology, AHA - American Heart Association, AMI - acute myocardial infarction, AST - aspartate aminotransferase, CK - creatine kinase, CK-MB - creatine kinase-muscle-brain, CV - coefficient of variation, cTnI - cardiac Troponin I, cTnT - cardiac Troponin T, ECG - Electrocardiogram, ESC - European Society of Cardiology, FAB - fragment antigen-binding, LDH - lactate dehydrogenase, LoD - limit of detection, hs-cTnT - high-sensitivity cardiac Troponin T, MI - myocardial infarction, NHLBI - National, Heart, Lung, and Blood Institute, RIA - Radio-immune assay, WHF - World Heart Federation.

**INDEX TERMS:** myocardial infarction, cardiac markers, troponin, troponin assays, high-sensitivity troponin.

## **MYOCARDIAL INFARCTION (MI) DEFINITION AND CARDIAC MARKERS**

Heart disease is still recognized as the leading cause of death for people of most ethnicities in the United States, accounting for 1 in every 4 deaths annually. Coronary artery disease (CAD) is the most common type of heart disease, accounting for the majority of deaths due to heart disease. CAD refers to a group of diseases including stable angina, unstable angina, and myocardial infarction (MI). An estimated 735,000 individuals suffer an MI annually, with the majority (roughly 525,000) being first time heart attacks.<sup>1</sup> According to the CDC, heart disease costs the United States an average 1 billion dollars every single day.<sup>2</sup>

Acute myocardial infarction is a life-threatening condition characterized by cell death due to a significant and sustained lack of blood flow to the heart. It is usually a consequence of plaque formation in the coronary arteries (coronary artery disease), but to a much lesser extent, can be due to other obstructing mechanisms. If it involves plaque formation, it is a consequence of atherosclerosis, a disease in which there is plaque buildup in one or more arteries in the body. Although atherosclerosis is often considered a heart problem, it can affect arteries anywhere in your body (heart, brain, arms, legs, pelvis, kidneys, etc.). Over time, the plaque hardens, causes the artery to narrow subsequently reducing the flow of oxygen-rich blood to the body. Without getting the proper blood that it needs, the affected organ/tissue ceases to function. When the myocardium does not receive the proper blood it needs to function it can lead to what is known as an MI. If too much of the myocardium is compromised by the MI, the heart ceases to function entirely, leading to death.<sup>1</sup>

John Hunter, an English physician in the eighteenth century, was one of the first physicians practicing Western medicine to describe the signs and symptoms of myocardial ischemia and the resulting MI. Although the history of MI and sudden death dates back much

further than that, it wasn't until the twentieth century when MI became well known as a cause of death. For centuries, MI was diagnosed based on patient symptoms alone, mainly due to a lack of understanding of the cause of MI and the lack of diagnostic tests used to aid in its identification.<sup>3</sup> It wasn't until the early 1900's when Willem Einthoven invented the string galvanometer, a device capable of recording the electrical activity associated with each heartbeat. Einthoven paved the way for the modern electrocardiogram, known to most as the ECG. In the 1920s, Sir Thomas Lewis ensured that the ECG was a staple in the clinical setting when evaluating the heart for CAD – identifying MI based on the electrical irregularities. For decades after, MI was strictly diagnosed based upon symptoms and disruptions of electrical activity (ECG changes).<sup>3</sup>

By the 1940s, cardiovascular disease had become the number one cause of mortality among Americans, accounting for 30-50% of deaths at that time. Prevention and treatment were so poorly understood that most Americans accepted early death from heart disease as unavoidable. Early death from heart disease became a national focus when, in 1948, President Truman signed into law the “National Heart Act.” In this act, US congress described heart disease as a condition seriously threatening the nation's health and allocated a \$500,000.00 seed grant for a twenty year epidemiological study and the establishment of the National Heart Institute, now known as the National, Heart, Lung, and Blood Institute (NHLBI). The twenty-year epidemiological study became known as the Framingham Heart Study (FHS).<sup>4</sup> The original FHS cohort included 5,209 male and female residents between the ages of 28-62 years from Framingham, Massachusetts. The overarching goal of the study was to identify the common factors that contribute to cardiovascular disease by following its development over a long period of time in a large group of participants who had not yet developed overt symptoms of the disease or suffered a heart attack or stroke.<sup>4</sup> In order to achieve this goal, the researchers followed these

subjects every two years over a period of several decades collecting valuable information through bi-annual medical histories, physical examinations, and laboratory tests.<sup>4</sup>

Since the initial assessment of the original cohort in 1948, the Framingham study has branched into several additional cohorts in 1971, 1994, 2002, and 2003, that have added valuable information/data to cardiovascular science and epidemiological research.<sup>4</sup> Many of the findings from the FHS are still in use today, for example, the results from the FHS led to the first description of the term, “risk factors.” These risk factors (high blood pressure, high blood cholesterol, smoking, obesity, diabetes, family history, and physical inactivity) are still in use in clinical practice and research today.

Soon after identifying risk factors for the development of cardiovascular disease, clinicians began to realize that there was a need to develop a better method of defining and diagnosing those presenting with MI. In 1954, the enzyme, aspartate aminotransferase (AST), was identified as being a useful cardiac biomarker.<sup>5</sup> Shortly after, total creatine kinase (CK) and lactate dehydrogenase (LDH) were added to the list of helpful enzymes in the detection of MI. Although these tests were readily available, they lacked specificity for AMI because all of the enzymes were also found in many other tissues and organs throughout the body. Due to this lack of specificity, these biomarkers carried little clinical significance by themselves. It wasn't until 1979 when the WHO recommended the panel of CK, AST and LDH for the diagnosis of AMI.<sup>6</sup> In late 1970's through the late 1980's, there was a major breakthrough with the identification and characterization of the isoenzymes of CK (CK-MM, CK-MB, CK-BB) and LDH (LDH1, LDH2, LDH3, LDH4, and LDH5). The CK-MB isoenzyme was identified and described as cardiac specific<sup>7</sup> and the electrophoretic analysis of CK-MB became popular followed by the utilization of mass assays to quantify CK-MB. Regarding the isoenzymes of LDH, normally LDH2 is

greater than LDH1. However, researchers found that those suffering from MI would present with a higher LDH1 fraction leading to what was known as an LD “flipped ratio.” Through the next 10-20 years advancements in cardiac markers were made such as the addition of myoglobin and its ability to pick up infarction within 12-24 hours, however, it wasn’t until the late 1980’s when the troponin assays not only revolutionized MI diagnosis, but brought to the forefront the importance of troponins in diagnosing AMI.

Even though clinical symptoms, electrocardiographic, laboratorial and imaging factors have all been taken into consideration when formulating the diagnosis of MI over the years, the current and Third Universal Definition of MI states that the term MI should be used when there is evidence of myocardial necrosis in a clinical setting consistent with myocardial ischemia.

Under these conditions any one of the following criteria meets the diagnosis of MI:

- Rise and/or fall of hs-cTn with at least one value above the 99<sup>th</sup> percentile upper reference limit (URL) and with at least one of the following: a. Symptoms of ischemia, b. New or presumed new significant ST-segment –T wave (ST-T) changes or new left bundle branch block (LBBB), c. Development of pathologic Q waves in the ECG, d. Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality, e. Identification of an intracoronary thrombus by angiography or autopsy
- Cardiac death with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes or new LBBB, but death occurred before cardiac biomarkers were obtained, or before cardiac biomarker values would be increased
- Percutaneous coronary intervention (PCI) related MI is arbitrarily defined by elevation of hs-cTn (>5 x 99<sup>th</sup> percentile URL) in patients with normal baseline



values ( $\leq$  99<sup>th</sup> percentile URL) or a rise of hs-cTn values  $>20\%$  if the baseline values are elevated and are stable or falling. In addition, either a. symptoms suggestive of myocardial ischemia or b. new ischemic ECG changes or c. angiographic findings consistent with a procedural complication or d. imaging demonstration of new loss of viable myocardium or new regional wall motion abnormality are required.

- Stent thrombosis associated with MI when detected by coronary angiography or autopsy in the setting of myocardial ischemia and with a rise and/or fall of cardiac biomarker values with at least one value above the 99<sup>th</sup> percentile URL.<sup>8-11</sup>

Through the advances of imaging and laboratory tests, it is now possible to detect ischemia causing necrosis of  $<1\text{g}$  of cardiac tissue.<sup>10</sup> Therefore, the general definition of MI has been adjusted through the years to follow the advances in technology and more emphasis has been placed throughout the revised and updated diagnosis recommendations of MI on other factors (symptoms, ECG, lab values, or imaging).<sup>9-11</sup> One of the factors with the highest degree of technological improvement, with substantial increase in specificity and sensitivity, are the assays targeting the cardiac troponins.

## **TROPONIN ASSAYS**

In terms of clinical importance and due to the isoforms of troponin available to separate skeletal muscle from heart muscle, assays for measuring Tn are designed to measure either cTnI or cTnT because these are the isoforms unique to cardiac muscle.<sup>8</sup> Troponins are released in the blood after myocardial injury in the form of complexes (ternary complex T-C-I, binary complexes I-C and I-T) and as free forms of troponin I and T.<sup>8,12</sup> The various commercially available cTn assays use capture and detection antibodies that target different epitopes. In these

assays, the detection antibody tag can be for example alkaline phosphatase, acridinium, fluorophor, europium, gold particles, ruthenium, or chemiluminiscence.<sup>12</sup>

The accurate and rapid evaluation of patients with acute coronary syndrome has always been a top priority for physicians and other healthcare providers due to the morbidity and mortality associated with MI and the cost of interventional strategies when they are not justified. The advancement in cardiac troponin assays has provided an opportunity for clinicians to more quickly identify acute coronary disorders and to determine the best treatment protocol for patients. Troponin studies have come a long way from the first identification of its isoforms in 1971, to current high sensitivity assays known as the fifth generation assays. The most recent troponin assays are capable of detecting troponin at concentrations 10-100 fold lower than earlier generations.<sup>13</sup> Each generation of troponin assay brought its own unique clinical significance and triggered protocols aiding in the differential diagnosis of coronary versus non coronary diseases.

When we examine the history of troponin as a cardiac biomarker, it is imperative that we also look at the assays of each generation beginning with the first to fifth generation. The first assay to measure troponin was identified in 1987 and measured TnI by radioimmunoassay (RIA).<sup>5</sup> This methodology utilized polyclonal rabbit antiserum, required two working days for the assay to be performed, and was capable of detecting troponin I levels as low as 10 ng/mL. This first generation assay captured cTnI levels that were elevated within 4-6 hours in patients with AMI. It peaked at levels of 112 ng/ml (range 20-550 ng/mL) at 18 hours and remained above normal value for up to 8 days following myocardial injury.<sup>5</sup> The first generation immunoassay assay for cTnT was developed by Katus and his colleague in 1989. It was based on an ELISA with two antibodies: the capture antibody conjugated to biotin (M7) and the detection antibody conjugated to horseradish peroxidase (IBIO).<sup>5</sup> This assay automatized in 1992 by its

incorporation onto the ES-analyzers (Boehringer Mannheim TM) had two problems. The first was due to the assay formulation which comprised a cardiac-specific capture antibody (with <0.5% cross-reaction to skeletal muscle) and a detection antibody that was only 78% cardiospecific. The 20% cross-reactivity of the second antibody resulted in falsely elevated TnT levels in patients with massive skeletal muscle damage (rhabdomyolysis). The second problem had to do with the assay turn-around time. According to *Danese et al. (2016)*, certain analyzers using the first generation assay took up to 90 minutes to complete which was hence inadequate to fulfill requirements for emergency testing.<sup>5</sup> Clinicians have recommended a turnaround time of 60 minutes or less from the time of blood collection to the reporting of results through the laboratory information system.<sup>14</sup>

The second generation Tn assays were developed in 1997. There were little improvements made such as the introduction of troponin antibodies M11.7 as a capture antibody and M7 as a detection antibody. This allowed for the correction of the non-specific binding to skeletal muscle troponin. With this new development, the normal range for troponin was between 0 and 0.1 µg/L. The limit of detection (LoD) and linearity of this assay were <0.05 and 12 µg/L, respectively.<sup>5</sup>

The third generation assays were introduced in 1999. The difference between the second and third generation assays was the use of human recombinant cTnT for calibration instead of bovine cTnT (second generation), which was thought to have considerably improved the assay linearity (inter-assay coefficient of variation <10% at 0.1 microgram/L).<sup>5</sup>

The fourth-generation cTnT assay was introduced in 2007. This assay used fragment antigen-binding (FAB) of two cTnT-specific mouse monoclonal antibodies in a sandwich format. The antibodies recognized two epitopes located in the central part of the cTnT molecule. The

fourth-generation cTnT assay has a LoD of 10 ng/L and a 10% coefficient of variation (CV) at 30 ng/L.<sup>5</sup>

In reference to its collection protocol and sensitivity of the assays, the first to the fourth generation troponin assays are consistent with the collection methods of earlier troponin testing. Sample requirement included serum, plasma or heparin samples.<sup>14</sup> As troponin assays with a higher analytical sensitivity have evolved, for example the fifth generation assays, we are now capable of detecting troponin levels (>99<sup>th</sup> percentile) in people with overt necrosis, micronecrosis, and also in 50% of healthy people. That is in stark contrast to all of the prior generation assays that were only capable of detecting those with overt necrosis (Table 1).<sup>15</sup>

## **THE 5<sup>TH</sup> GENERATION OR HIGH SENSITIVITY TROPONIN ASSAY**

The fifth generation assays, known as high sensitivity troponin (hs-cTn) assays, are defined as high sensitivity assays if they fulfill the following two conditions: 1) a coefficient of variance less than 10% at the 99<sup>th</sup> percentile value of the reference healthy population, and 2) concentrations above the assay's limit of detection are measurable in greater than 50% of healthy individuals. The fifth generation assays are modifications of the fourth-generation assay and were utilized outside of the US beginning in 2010. For the fifth generation assay the biotinylated capture antibody was not changed, whereas the detection antibody was genetically re-engineered into a mouse-human chimeric detection antibody to reduce the susceptibility of interference by heterophilic antibodies. In hs-cTn assays, cross-reactivity should be less than 0.1%.<sup>8</sup> The analytical sensitivity of this generation assay was improved by increasing the sample volume from 15 to 50  $\mu$ L, increasing the ruthenium concentration of the detection antibody, and lowering the background signal through buffer optimization. As a result, the analytic performance of the hs-cTnT assay had been significantly improved: the LoD was 5 ng/L, the

99th percentile cutoff point was 14 ng/L, and the CV was 10% at 13 ng/L.<sup>5</sup> As expected, the improved sensitivity of the new generation immunoassays came along with a decreased specificity for AMI as this assay is so sensitive it is capable of being detected in 50% of healthy people. The fifth generation assay further emphasizes the true clinical role of troponins which is an organ specific marker not disease specific marker. Measurable hs-cTn values can now be found in several non-ischemic cardiac conditions, including, among the other, atrial fibrillation, hypertension, renal and liver disorders, acute or chronic pulmonary disease and even severe allergic reactions as well as 50% of the normal population. Therefore, careful clinical assessment, serial testing and thoughtful differentiation are required to separate AMI from other acute and chronic disorders which can be associated with low-level and less harmful myocardial injury.<sup>5</sup>

Due to the current diagnosis of MI being based on the rise and fall of troponin (with at least one measurement being above the 99<sup>th</sup> percentile Upper Reference Limit (URL)) with strong clinical evidence, great debate exists around what should constitute a normal population from which the 99<sup>th</sup> percentile cut off will be calculated and based. Issues revolve around what assessments should be done to make sure the population is truly free of cardiovascular disease, and how many people should be included in those calculations (sample size).<sup>8</sup> Male gender, increased age, specimen used and the testing method, have all been identified as potential factors which affect the troponin cut-off calculations.<sup>8</sup>

As we take a closer look at the high sensitivity troponin assay, we need to take into consideration issues related to this assay in all steps of the testing process (pre-analytical, analytical, and post-analytical). In the pre-analytical phase an important consideration is the timing of the specimen collection. According to the most current guideline for the definition and

diagnosis of AMI, blood samples should be collected at presentation and repeated at 3 and 6 hours for fourth generation troponin assays.<sup>8,9</sup> During the analytical phase, the coefficient of variation (CV) of the assay needs to be taken into consideration when choosing troponin assays. Coefficient of variation relates to the assay precision. The optimal coefficient of variation is  $\leq 10\%$ , and assays with coefficient of variation  $>20\%$  should be avoided.<sup>8,9</sup>

In addition to pre-analytical and analytical phase considerations, post-analytical phase or interpretation of troponin values considerations also exist. High sensitivity troponin assays make it possible to detect troponin levels in healthy individuals ( $\geq 50\%$  of healthy individuals) versus  $\leq 20\%$  with contemporary assays hence the importance of the coefficient of variation and serial testing.<sup>8</sup> One set back of the high sensitivity troponin assay is that there is no equivalence across different assays (lack of standardization).<sup>8</sup> Besides the analytical variation across assays, there is also variability within the same subject (biological variability).<sup>8</sup> Another consideration is that since differences in values of troponin in males and females are observed, with female values being lower than males, gender specific values might be more appropriate when interpreting results of high sensitivity troponin assays.<sup>8</sup> There are other health conditions besides MI that can present with elevated troponin levels and should be considered in the differential diagnosis such as heart failure, renal failure, and arrhythmias.<sup>8,9</sup>

High sensitivity troponin assays were FDA approved for clinical use in the US in 2017. The availability and uptake into clinical practice of this highly sensitive troponin assay and revised diagnosis of MI has implications for providers, patients and community.<sup>8-11</sup> Providers need to be familiar with the new technology and the interpretation of the lab results where the units of measure are changing from ng/mL to ng/L. For example, as a result of the improved sensitivity of the new assay, patients that have been previously diagnosed as having different

forms of angina, now meet the diagnostic definition of MI. This has also implications for the patient in many aspects including health insurance qualification, work adjustments, licensing for driving or piloting, besides the psychological burden of an MI diagnosis. On a community level, changes in disease definition with more people being diagnosed, have implications on the recorded number of cases of MI which is used to allocate resources and to evaluate efficacy of intervention measures.<sup>8-11</sup> On a global scale, health care facilities in resource limited countries that cannot afford these new advanced technologies such as Troponin assays will need to use less sensitive and less specific methods, and will follow the WHO Category C definition and diagnosis criteria of probable MI.<sup>9-11</sup>

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Table 1. Detection Capability of Troponin Assays in Different Clinical Presentations of Myocardial Infarction.

Troponin Assay	Clinical Presentation		
	Normal	Micro-necrosis	Overt Necrosis
Generation 1-3			√
Generation 4			√
Generation 5	√	√	√