REVIEW ARTICLE

Biological Markers of Myocardial Necrosis

Miguel Santaló Bel,[a] Josep Guindo Soldevila,[b] and Jordi Ordóñez Llanos[c]

[a]Complejo de Urgencias, Emergencias y Críticos, Hospital de la Santa Creu i Sant Pau, Universidad Autónoma, Barcelona. [b]Servicio de Cardiología, Hospital de la Santa Creu i Sant Pau, Universidad Autónoma, Barcelona. [c]Servicio de Bioquímica, Hospital de la Santa Creu i Sant Pau, Universidad Autónoma, Barcelona, Spain.

New biological markers of myocardial injury have improved the management of patients with acute coronary syndromes.

Among these markers, the most relevant are the cardiac troponins (troponin I and troponin T) because of their cardiospecificity, and myoglobin because of its combination of diagnostic sensitivity and usefulness for an early diagnosis. The serial analysis and combined use of both markers fulfill all diagnostic and prognostic requirements, and are helpful in indicating therapeutic strategies for acute coronary syndromes. However, these markers also have limitations, and their concentrations should always be interpreted in the light of the patient's clinical status.

Key words: *Acute coronary syndromes. Troponin. Myoglobin.*

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Marcadores biológicos de necrosis miocárdica

La aparición de los nuevos marcadores biológicos de daño miocárdico, especialmente troponinas y mioglobina, ha supuesto un notable avance en el manejo de los pacientes con síndrome coronario agudo.

Entre los marcadores biológicos de daño miocárdico destacan de manera especial las troponinas cardíacas (TnTc o TnIc), por su cardioespecificidad, y la mioglobina, por su combinación de sensibilidad y precocidad diagnóstica. El análisis seriado y el uso combinado de ambos marcadores permite cubrir las necesidades diagnósticas, pronósticas y de indicación terapéutica del síndrome coronario agudo. Sin embargo, a pesar sus indudables ventajas, hay que enfatizar la importancia de conocer sus limitaciones e interpretar sus resultados teniendo siempre muy en cuenta el contexto clínico de paciente.

Palabras clave: *Síndrome coronario agudo. Troponina. Mioglobina.*

INTRODUCTION

Cardiovascular diseases are the leading cause of death in Spain, both in men and women. In addition, they account for high morbidity, so that their impact on health and social services (the need for costly and limited clinical and therapeutic resources) and their socioeconomic relevance (cause of temporary or permanent disability, impairment, etc.) are high. Among cardiovascular diseases, ischemic heart disease is the leading cause of death in men and the second most frequent cause in women. Many such deaths from coronary causes occur during the decompensatory phase of coronary arteriosclerosis commonly known as acute coronary syndrome (ACS).

The severity of ACS and its resulting morbidity and mortality depend largely on whether or not myocardial necrosis occurs. In diagnosing myocardial necrosis, clinical symptoms and the results of electrocardiography (EKG) are important, yet the final diagnosis is often based on the results of tests for its biologic markers.

Just one decade ago, the only biologic markers for myocardial necrosis that existed were the catalytic activity of total creatine kinase (CK) or of its more cardiospecific isoenzyme, creatine kinase MB (CK-MB). However, none of these classic markers has the diagnostic specificity needed to adequately meet new clinical requirements that have arisen over time.

Since the early eighties, the panel of biologic markers for myocardial necrosis has changed considerably. It was during those years that immunoassays were developed, making it possible to quickly determine CK-MB or myoglobin levels and thus to rapidly diagnose ACS. Around the same time, isoforms of CK isoenzymes were also assessed for their usefulness in diagnosing myocardial necrosis (particularly CK-MB isoenzymes). They contributed significantly to early

Correspondence: Dr. J. Guindo Soldevila.
Unidad Coronaria. Servicio de Cardiología.
Hospital de la Santa Creu i Sant Pau. Universitat Autònoma de Barcelona.
Avda. Sant Antoni Maria Claret, 167. 08025 Barcelona. España.
E-mail: jguindos@hotmail.com

diagnosis, but not to diagnostic specificity. The first methods used to measure cardiac isoforms of T and I troponins began to appear at about the same time, and the results obtained for ACS raised doubts as to their specificity. Nonetheless, today these doubts have been completely dispelled, and cardiac troponins remain the diagnostic pillars on which clinical management, risk stratification, and the treatment of many ACS are based.

The role of cardiac troponins has been so crucial in assessing ACS, that in 2000 the European Society of Cardiology and the American College of Cardiology, based on the guidelines developed in 1999 by the U.S. National Academy of Clinical Biochemistry, jointly redefined myocardial infarction. Under this redefinition, biochemical markers play an even more important role than in the definition that was drawn up in 1971 by the World Health Organization (OMS, 1971).

It would appear, in light of the above, that measuring cardiac troponin levels would lead to detection of myocardial necrosis in 100% of ACS patients, yet there are methodological problems that one must be aware of and take into account in order to correctly interpret these biologic markers. This review takes a comprehensive look at the most important biochemical, methodological, and clinical issues surrounding the current role of markers of myocardial necrosis under the new definition of myocardial infarction.

BIOLOGIC MARKERS OF MYOCARDIAL NECROSIS

Release of molecules from the necrotic myocardium

Of the products released from the cell during ischemia/necrosis, those that are a solute in the cytoplasm and smaller in size are the ones that can most easily reach the circulation. For this reason, they are the earliest markers of cellular damage. Such markers are most often ions and sometimes metabolites, such as lactate. Since they are found in all body tissues, the arrival in the plasma of intracellular metabolites, such as lactate, cannot be interpreted as being specifically the result of a cardiac insult. If the insult persists, the damaged cell will release cytoplasmic macromolecules, most of them enzymes having higher cardiospecificity, such as creatine kinase, lactate dehydrogenase, aspartate aminotransferase, or myoglobin. If the cellular damage persists and necrosis appears, structural macromolecules will be released into the plasma. Despite some debate, detection of even small quantities of intracellular structural proteins (mitochondrial, nuclear, or from the cell's contractile apparatus) is always indicative of irreversible necrosis.

The probability that a cardiac marker will turn out positive in a patient with myocardial necrosis depends on how it is released from cells and cleared from the plasma; on the time elapsed between its measurement and the onset of the myocardial damage, and on the properties of the testing method (particularly how sensitive and inaccurate it is). Elevated blood levels of sensitive and specific markers for myocardial necrosis do shed light on the pathogenesis of the process. In the clinical presence of acute ischemia, a rise in a sensitive and specific marker above the reference limit signals the presence of acute myocardial infarction (AMI) (see the redefinition of AMI further down). If cardiospecific markers are elevated in the absence of ischemic heart disease, one must look for other pathogenic mechanisms as the cause of the myocardial necrosis or rule out the possibility of a false positive result (Table 1).

The role of biologic markers in detecting myocardial necrosis

Biologic markers of myocardial damage have played an essential role in determining the diagnosis, prognosis, and risk stratification of patients with ASC. Until very recently, a diagnosis of AMI was based on the presence of at least two of the three following criteria, which were established by the World Health Organization (WHO) in 1971:[1] ischemic chest pain, EKG changes suggestive of ischemia, a rise in plasma or serum CK or CK-MB catalytic activity. However, a significant portion of patients with AMI have atypical clinical symptoms or may not have symptoms of myocardial ischemia at all.[2] On the other hand, despite the unquestionable usefulness of EKG, 30% of patients with AMI have EKG tracings within the normal range or changes that are non-diagnostic or hard to interpret, thus making diagnosis difficult.[3] Consequently, measuring biologic markers of myocardial necrosis has

TABLE 1. **Potential reasons for a rise in cardiac troponin levels**

Myocardial infarction
Injuries
Myocardial contusion
Pacemaker
Heart surgery
Heart failure
Hypertensive cardiomyopathy
Hypotension
Severe tachycardia or bradycardia
Pulmonary embolism
Cardiomyopathy associated with advanced renal failure
Diabetes mellitus
Myxedema coma
Myocarditis
Post-angioplasty
Sepsis
Amyloidosis
Acute neurologic disorder

TABLE 2. **Features of the main biochemical markers for myocardial necrosis**

Ideal features	Total CK	CKMBa	CKMBm	MYO	TnT	TnI
Easily measured	Yes	Yes	Yes	Yes	Yes	Yes
Tests available	Yes	Yes	Yes	Yes	Yes	Yes
Fast results	Yes	Yes	Yes	Yes	Yes	Yes
High specificity	No	No	No	No	Yes	Yes
Sensitivity for micro AMI	No	No	No	No	Yes	Yes
Sensitivity for early AMI	No	No	No	Yes	No	No
Sensitivity for advanced AMI	No	No	No	No	Yes	Yes
Low cost	Yes	Yes	No	No	No	No
Point-of-care systems available	Yes	No	Yes	Yes	Yes	Yes

CK indicates creatine kinase; CKMBa and CKMBm, catalytic activity and mass concentration of creatine kinase, respectively; MYO, myoglobin; TnT and TnI, cardiac isoforms of troponin T and I; POC, point of care systems.

been and continues to be crucial in diagnosing AMI.

Biologic markers, despite their usefulness in arriving at a conclusive diagnosis of AMI, still have two disadvantages:

–They can only identify patients with myocardial necrosis from among patients with ACS. Even though methodologies are being developed and validated for identifying myocardial ischemia, as yet such methodologies cannot be applied in a clinical setting.[4-6] Thus, the diagnosis of unstable angina (UA) remains only clinical and has all the limitations mentioned so far. For this reason, a correct diagnosis can be made only if ischemia is induced through controlled stress tests.

–A certain amount of time must have elapsed before abnormal elevations can be detected. However, morbidity and mortality from ACS are lower the earlier treatment is initiated. For this reason, new markers or strategies for detecting myocardial necrosis or, better still, myocardial ischemia must be developed as quickly as possible.

From 1954, when aspartate aminotransferase activity was first measured in assessing myocardial necrosis, to the present, the number of biologic markers for this enzyme has increased remarkably. Over time we have progressed from markers having poor sensitivity and specificity to those in current use, which can pick up small areas of myocardial necrosis. As mentioned earlier, it is still not possible to detect the changes that precede myocardial necrosis by means of biologic markers for ischemia, yet new markers (cardiac troponin, myoglobin, or CK-MB levels) meet many of the clinical requirements for assessment, diagnosis, risk stratification, and treatment guidelines in patients with ACS. The most important features of the markers that are most widely used today will be described in the following pages.

There are several biologic markers for myocardial necrosis, all having different properties and diagnostic value. All of them are proteins, and the most widely used today and up to now in clinical practice are enzymes, such as total creatine kinase (CK) and its cardiac isoenzyme (CK-MB), and isoforms of CK-MM (CK-MM1, CK-MM2, and CK-MM3) and of CK-MB (CK-MB2 and CK-MB1), and products other than enzymes, such as cardiac troponins T and I (cTnT and cTnI). Tables 2 and 3 show the various features of these markers, with the exception of isoforms of CK-MM and CK-MB.

«Classical» biologic markers

Total creatine kinase

Until other markers became available, total CK was the biologic marker most widely used to diagnose myocardial and musculoskeletal changes. Today it still plays an important role in follow-up during the subacute phase of a myocardial infarction. CK (whose molecular weight is 85 kDa) is an enzyme that is present in virtually all body tissues, since it catalyzes a reaction involving energy transfer, namely the conversion of creatinine into creatine phosphokinase through phosphorylation. In cells it is present mainly in the cytoplasm. CK is most abundant in striated muscle, which is the reason that its reference values depend on muscle mass and are higher in men than they are in women. When there is myocardial necrosis, the catalytic activity of CK can be detected above the upper reference limit beginning 4-6 hours after the first symptoms. Total CK is not a cardiospecific molecule and its reference values vary broadly depending not only on muscle mass, as mentioned earlier, but also on age (the higher the age, the lower the value), race (its activity is higher in blacks), and physical activity (it rises after activity, in direct proportion to the length of the activity and its intensity, and in inverse proportion to the degree of prior training).[7] Furthermore, CK levels can rise in a broad spectrum of pathologic conditions,[8,9] even without myocardial necrosis.

TABLE 3. **Advantages, disadvantages, and recommendations connected with the use of the main markers of myocardial necrosis**

Marker	Advantages	Disadvantages	Recommendations
Total CK	Fast results Tests available Can detect early infarctions	Low cardiospecificity Low availability Not very useful for predicting cardiovascular risk	Recommended only if CKMB or troponin levels are unavailable
CK-MB (activity)	Fast results Tests available	Cardiospecificity low but higher than that of total CK Low sensitivity Can detect early infarctions	Recommended only if CK-MB or troponin levels are unavailable
CK-MB (mass)	Fast results Tests available Can detect early infarctions	Cardiospecificity low but higher than that of total CK Low sensitivity Not very useful for predicting cardiovascular risk	Use as an alternative option if troponins are unavailable
Myoglobin	Fast results Availability of tests Can detect early infarctions Can detect reperfusion POC systems available	Low cardiospecificity Low overall sensitivity for infarction (unable to detect small infarcts) Not very useful for predicting cardiovascular risk	Do not use as a single marker
Troponins	Fast results Tests available Improved diagnostic sensitivity Cardiospecificity Can predict cardiovascular risk Of limited usefulness in detecting reinfarction POC systems available Useful as a single marker	Not commonly used in clinical practice Of limited usefulness in detecting early infarctions It should be measured as currently ecommended Of limited usefulness in detecting very early infarctions (<3 h)	Useful in guiding therapy

Creatine kinase MB (CK-MB)

Isoenzymes are specially adapted forms of the enzymes that are found in various cells and tissues. CK isoenzymes are made up of groups of monomers. There are three CK isoenzymes, each of which is made up of two monomers, M and B, which are in turn grouped in dimers, thus making up the functional enzyme. CK-MM (homodimer of the M monomer) is mostly found in striated skeletal muscle — 95% of all CK is CK-MM —, and CK-MB (heterodimer of monomers M and B) is more plentiful in the myocardium (up to 20% of total CK in a damaged myocardium has been described as CK-MB, although this percentage is lower in a healthy myocardium).[10] There is a third isoenzyme, the homodimer of monomer B, known as CK-BB, that is primarily located in the central nervous system and the intestines.[11]

Thus, CK-MB would appear to be the most cardiospecific of all the enzymes that make up what is known as total CK. Still, CK-MB is also found in small amounts in skeletal muscle (around 5% of all CK activity is from CK-MB), although the amount can rise under certain physiologic conditions (heavy physical exercise, such as in marathon runners), disease states (genetic or acquired myopathies),[8,12] and even some non-muscular problems, such as certain neoplasms.[8,13] For these reasons, the presence of extramyocardial «background noise», which may be physiologic or pathologic, from the circulating catalytic activity of CK-MB in the plasma of healthy individuals limits its diagnostic usefulness for assessing myocardial necrosis. Another important factor that limits the diagnostic value of CK-MB levels is the in vivo and in vitro interference of methods used to assess its catalytic activity, as a result of which catalytic activity can appear to be falsely elevated. Macrokinases[14] or nonspecific kinases, since they lead to such false elevations of plasma catalytic CK-MB activity, can give rise to CK-MB levels that are compatible with myocardial infarction in patients who have not had a heart attack.

A simple way to improve the cardiospecificity of CK-MB determinations is to express the results as a quotient of the total catalytic activity of circulating CK. In this way, a plasma level above the CK-MB fraction normally found in skeletal muscle can be taken as a sign that the isoenzyme is being released from the myocardium. However, the quotient given by CK-MB

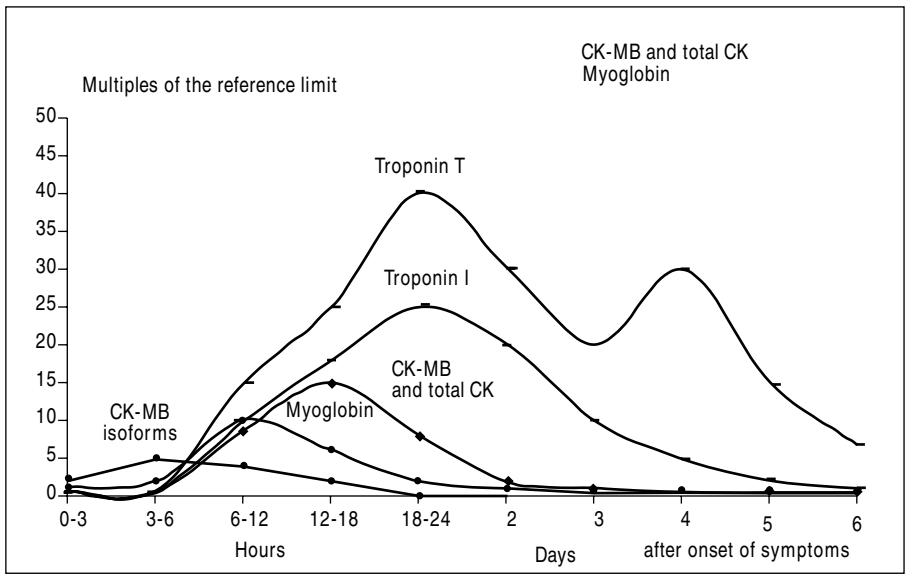

Fig. 1. Changes over time in biochemical markers for myocardial necrosis after myocardial infarction.

divided by total CK (CK-MB/total CK) is also far from ideal in that it lacks the combined diagnostic sensitivity and specificity currently required to diagnose a myocardial infarction.

Most problems arising from the methods used to assess the catalytic activity of CK-MB have been solved by determining its mass concentration. For this reason, in addition to their higher sensitivity and accuracy, immunoassays for measuring the mass concentration of CK-MB have displaced determinations of its catalytic activity. The mass concentration of CK-MB varies depending on the type of immunoassay used to measure it, although an international standard is being developed that will make the results transferable across methods. Thus, it is advisable to obtain reference values for the mass concentration of CK-MB in each laboratory. As in the case of catalytic activity, using the ratio given by the concentration of CK-MB against total CK catalytic activity (CK-MB concentration/total CK catalytic activity) improves cardiospecificity.[15]

CK-MB activity/concentration can show elevated plasma levels beginning 4-6 hours after initial symptoms of AMI and remain elevated up to 24-36 hours after the onset of symptoms[16-18] (Figure 1). Because of this fast rise and fall, CK-MB can be used to detect subsequent reinfarction. Like myoglobin and CK, the mass concentration of CK-MB is limited by its poor cardiospecificity; despite the fact that CK-MB isn't susceptible to the methodological interferences that affect catalytic activity, its plasma levels can rise under the same conditions causing a rise in catalytic activity, even in the absence of a myocardial lesion.[9] Since CK-MB is not an early marker of myocardial necrosis, levels found on admission are normal in 35% to 50% of patients with AMI.[19,20] Before the more recent markers of myocardial necrosis were developed, CK-MB played a crucial role in the diagnosis of AMI based on WHO criteria. Despite its limitations, CK-MB has been essentially the gold standard against which other biochemical markers of myocardial necrosis are compared.

CK-MB isoforms

Isoforms of CK-MM and CK-MB, resulting from posttranscriptional changes in CK isoenzymes, retain the enzyme's catalytic activity but have different molecular mass and physical and chemical properties.[21] In muscle (cardiac and skeletal) there is only one isoform of CK-MM and CK-MB (CK-MM3 and CK-MB2), which is the genetically encoded isoenzyme. After tissue necrosis, CK-MM3 and CK-MB2 are quickly released into the plasma, where they are rapidly converted to CK-MM2 and CK-MB1, respectively, by a carboxypeptidase (Figure 2).[22] Under normal conditions, tissue isoform variants of CK-MM3 and CK-MB2 are in equilibrium with their plasma isoforms (CK-MM2-CK-MM1 and CK-MB1), and the ratio between them (CK-MM3/CK-MM1 and CK-MB2/CK-MB1) is close to 1.0. Conversion from tissue isoforms to plasma isoforms takes place faster in the case of CK-MB2 than CK-MM3. During an AMI, the myocardium releases large amounts of CK-MB2 that cannot be completely converted to CK-MB1 in plasma; as a result, a ratio of CK-MB2/CK-MB1 that is ≥1.5 has high diagnostic sensitivity for myocardial necrosis, particularly 0-6 hours after onset.[23] By measuring CK-MB isoforms, nearly 100% (92%) of patients with myocardial necrosis can be detected within the first 6 hours after chest pain begins, even though their main diagnostic value lies in their high negative predictive value in connection with AMI. In a recent study, CK-

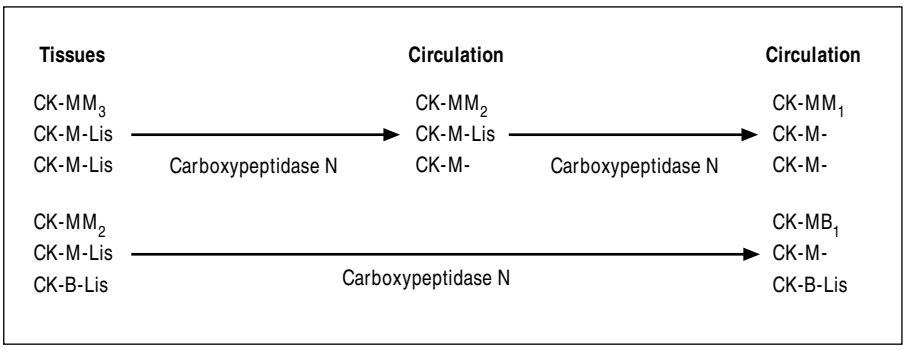

Fig. 2. *In vivo* generation of CK-MM and CK-MB isoforms.

MB isoforms were the most sensitive biologic markers (91%) in the early diagnosis (<6 hours) of AMI in patients with chest pain who were seen in emergency rooms.[24] Notwithstanding, isoforms of CK-MB, like total CK, CK-MB, and myoglobin, are not cardiospecific, since they are found in both skeletal and myocardial muscle.[25] On the other hand, levels are not so easily obtained, and there is much subjectivity in how results are interpreted. Despite its usefulness in early diagnosis, all these disadvantages explain why isoforms of CK-MB (and CK-MM) are seldom measured as part of the regular diagnostic workup for AMI.

Myoglobin

Myoglobin is a protein situated in the cytoplasm whose low molecular weight (18 kDa) enables it to get into the circulation quickly with moderate changes in cell permeability. Myoglobin is released soon after chest pain begins, and increased levels can sometimes be found one to two hours after the AMI has begun. Myoglobin reaches its highest levels in plasma from 6 to 12 hours after an AMI and disappears from the bloodstream 12-24 hours after the onset owing to its rapid clearance by the kidneys. Formerly, plasma myoglobin levels were obtained using radioimmunoassay methods that did not yield results quickly enough for the emergency diagnosis of AMI. Today, thanks to the use of monoclonal antibodies applied in non-radioactive immunoassays, myoglobin can be measured in minutes and can thus be used in the early diagnosis of AMI.[26] However, myoglobin levels have important limitations in connection with this diagnosis, mainly that no structural differences exist between the molecule expressed in myocardial and skeletal muscle, since the latter cells undergo normal turnover. Besides, the presence in plasma levels of trace levels of myoglobin (and of other molecules sharing similar properties) limits its cardiospecificity and usefulness in early diagnosis (Figure 3). Increased myoglobin levels are also found in patients with renal failure as a result of reduced clearance by the kidneys;[27] thus, myoglobin has low diagnostic efficiency in such patients at high risk for myocardial necrosis.[28] Finally, there are methodological factors that limit its diagnostic efficiency, since there is no single agreed-upon level that is indicative of myocardial necrosis and since myoglobin levels vary depending on the testing method used.

Myoglobin is useful primarily because of its high sensitivity and negative predictive value during the first hours after an AMI.[29,30] This means that by measuring myoglobin levels myocardial necrosis can be safely ruled out within the first 6 hours after the patient is admitted.[31,32] Still, myoglobin's poor cardiospecificity and fast renal clearance lower its positive predictive value and make it impossible to rely on a single finding of increased levels in making decisions. An isolated rise in myoglobin in a patient with non-diagnostic EKG results makes it necessary to seek another marker that is more cardiospecific.[33,34] Finally, myoglobin´s main diagnostic usefulness, owing to its fast release from cells and arrival in the bloodstream, lies in assessing the effectiveness of coronary reperfusion after thrombolytic therapy (Figure 4).

New biologic markers. Troponins

The troponin complex is located in the fine filament of the tropomyosin complex within contractile cells. Three different troponins are encoded by different genes:[35] troponin C, which binds to calcium; troponin I (TnI) or inhibitory molecule, which prevents muscle contraction in the absence of calcium, and troponin T (TnT), which binds to tropomyosin. Only TnT and TnI have any clinical significance, since they have cardiospecific isoforms (cTnT and cTnI) whose aminoacid sequence allows them to be distinguished immunologically from the skeletal isoforms.

Unlike myoglobin and CK isoenzymes, which are dissolved within the cytoplasm of the cell, most troponin is structurally bound to the tropomyosin complex, even though a small fraction (6%-8% of cTnT and 3%-8% of cTnI) is also dissolved in the cellular cytoplasm.36 The molecular weight of cardiac troponin (cTnI=22 kDa; cTnT=37 kDa) is similar to that of CK-MB. Such factors suggest that even though troponin is

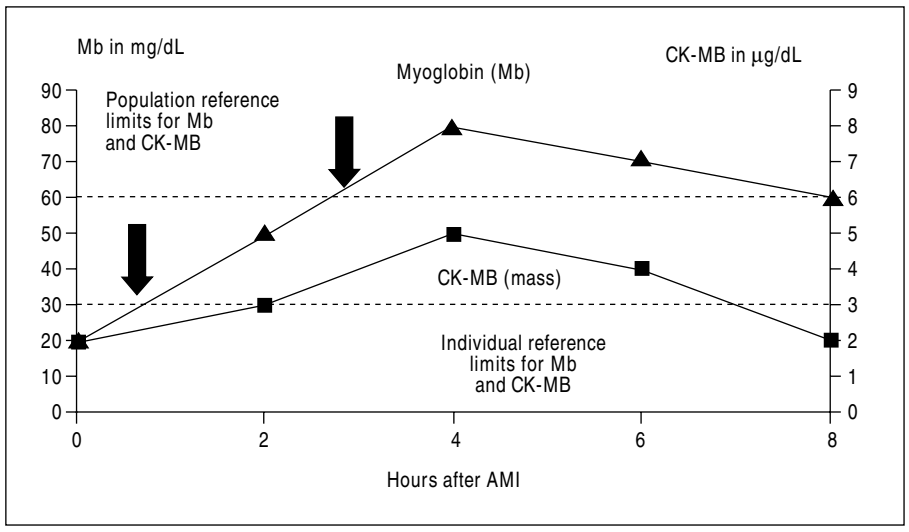

Fig. 3. «Biological background noise» affecting non-cardiospecific markers limits their sensitivity and usefulness in the early diagnosis of myocardial infarction.

predominantly a structural molecule, its cytoplasmic fraction should be released as early as CK-MB. This is confirmed by looking at the plasma kinetics of the different markers after an AMI (Figure 1).

When myocardial necrosis takes place, cardiac troponin is found in plasma 4-6 hours after the onset of symptoms, probably as a result of early release of its cytoplasmic component. Release of cTnT and cTnI follows different kinetic mechanisms. CTnT reaches an initial peak 12 hours after symptoms begin, plateaus over the first 48 hours, and gradually declines up to the 10th day. This makes it possible to diagnose an infarct subacutely. However, finding increased plasma levels (which vary from the 7th to the 21st day) will depend on how large the AMI is.[37] The kinetics of CTnI release are similar, only CTnI reaches a lower peak[38] than cTnT and returns to normal levels faster. As in the case of cTnT, however, how fast normal levels are attained will depend on the size of the AMI.

Top reference values for defining myocardial necrosis

In the absence of acute or subacute myocardial necrosis, plasma levels of cardiac troponins should be undetectable; the very low levels found in reference subjects are caused by methodological «background noise», not to myocardial necrosis. Consequently, cardiac isoforms are completely cardiospecific, unlike all other biologic markers for myocardial damage, a feature that makes it possible to detect small areas of myocardial necrosis, previously known as «minimal myocardial necrosis», and that has broadened this marker's diagnostic usefulness. In patients with classic

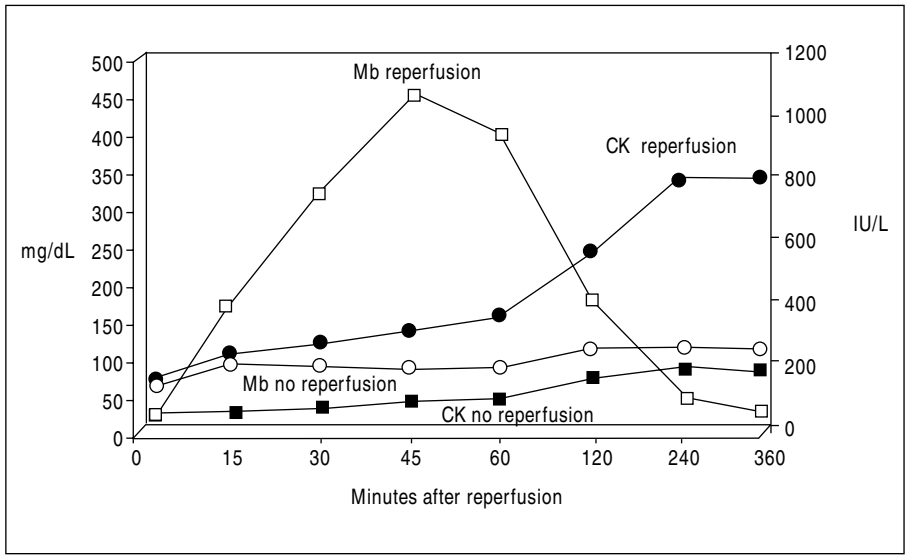

Fig. 4. Myoglobin: usefulness for assessing the course of myocardial reperfusion.

UA, cardiac troponin levels can reveal myocardial infarcts that are not picked up by other markers of myocardial necrosis.[39] In patients with non-cardiac conditions,[40] they can also reveal myocardial damage that worsens the patient's survival prognosis. However, such methodological «background noise», which results from the poor accuracy of the test when very small levels of cardiac troponin are found, leads to a loss of diagnostic sensitivity. Furthermore, the levels that are detectable in reference subjects vary among methods. Consequently, the type of cardiac troponin that is measured and the testing method used are decisive in interpreting the results and assessing their diagnostic value. Recent guidelines for the diagnosis of myocardial infarction that were developed by the European Society of Cardiology and the American College of Cardiology have established under what conditions upper reference values of troponin should be obtained so as to define the presence of myocardial infarction.[41,42] Any troponin level that is obtained when an ischemic syndrome is present and that lies above the 99th percentile of a reference population would define an AMI as long as the measurement has been made with an interserial analytical inaccuracy not to exceed 10%. This definition challenges the manufacturers of troponin tests, who should aim to increase their inaccuracy as much as possible. The greater the inaccuracy, the lower the threshold for detecting an AMI and the higher the ability to identify a small area of myocardial necrosis. In addition to this required level of inaccuracy, the Committee on Standardization of Markers of Cardiac Damage of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) stipulates that, to avoid interference from potential nonspecific effects, detection thresholds should be around 5 times lower than the clinical decision thresholds based on the aforementioned criterion.[43]

There is only one method for measuring cTnT, so that the results obtained in different laboratories can be considered homologous, and only one reference value for defining myocardial necrosis (and this must be obtained with a level of inaccuracy lower than 10%). In the case of cTnI, numerous methods (more than ten) exist that differ among themselves.[37] Even a test that has been developed by the same manufacturer can have different values for defining myocardial necrosis when applied to different instruments. Thus, cTnI results obtained through different methods cannot be considered homologous, and there is no single reference value for defining myocardial necrosis. By way of an example, this value can vary among methods by a factor of 20 (0.1-2.0 μg/L). Currently a reference material is being developed for nTcI that will make it possible to standardize and make transferable the results of different tests.

Release into the plasma

It has been shown that after a myocardial infarction the heart initially releases free cTnT into the plasma. Later, it releases free cTnI, tertiary cTnT-cTnI-TnC complexes, and, occasionally, some fragments of cTnT. Tertiary complexes have a short half life, since they are rapidly broken down into free cTnT and cTnI-TnC binary complexes.[44] In the case of cTnI, the form that is secreted in the greatest quantities is the cTnI-TnC binary complex, although free cTnI can also be secreted in either its oxidized or reduced form[45] (Figure 5). The proportions of cTnI and cTnI-TnC that are secreted after a myocardial infarction fluctuate over time, with greater amounts being secreted during the more advanced stages of the infarct. Once they are released into the plasma, cTnI and its complexes can be phosphorylated, dephosphorylated, or degraded through proteolysis. These multiple forms that circulate in the plasma widen the gap between the nTcI values obtained through various methods, since, as shown in Figure 6, different methods differ in their ability to recognize the various forms of nTcI.[44] It has been shown that the portion of the troponin molecule that remains most stable throughout all the changes lies between amino acids 30 and 110.[37] For this reason, the IFCC´s aforementioned Committee on Standardization of Markers of Cardiac Damage recommends using, for developing troponin tests, antibodies that target the epitopes located in the stable region of the molecule, since they are affected very little, if at all, by complex formation or other changes in vivo.[45]

Reexpression of cardiac troponin isoforms in skeletal muscle. Their relationship to cardiospecificity of cardiac troponin tests

Troponin T (TnT) is a molecule weighing 37 kDa that has three specific isoforms, one for each type of muscle fiber that exists (cardiac muscle and fast- and slowly-contracting skeletal muscle). The primary structures of isoforms differ among themselves enough that each isoform can be identified by means of immunoassay. During fetal development, cardiac and skeletal muscle isoforms are simultaneously expressed in both tissues; in adult life, expression of these isoforms becomes selective for each tissue.[46] However, in some animal models[47] and certain diseases of skeletal muscle (polymyositis and genetic muscular dystrophies or myopathy resulting from chronic renal failure) reexpression of some cardiac isoforms of TnT has been noted.[48] Reexpression of cardiac isoforms in skeletal muscle was found when many patients with end-stage renal failure were noted to have detectable plasma levels of cTnT, as opposed to a much smaller number that had elevated cTnI levels.[49] These results were obtained with early versions of cTnT tests, which

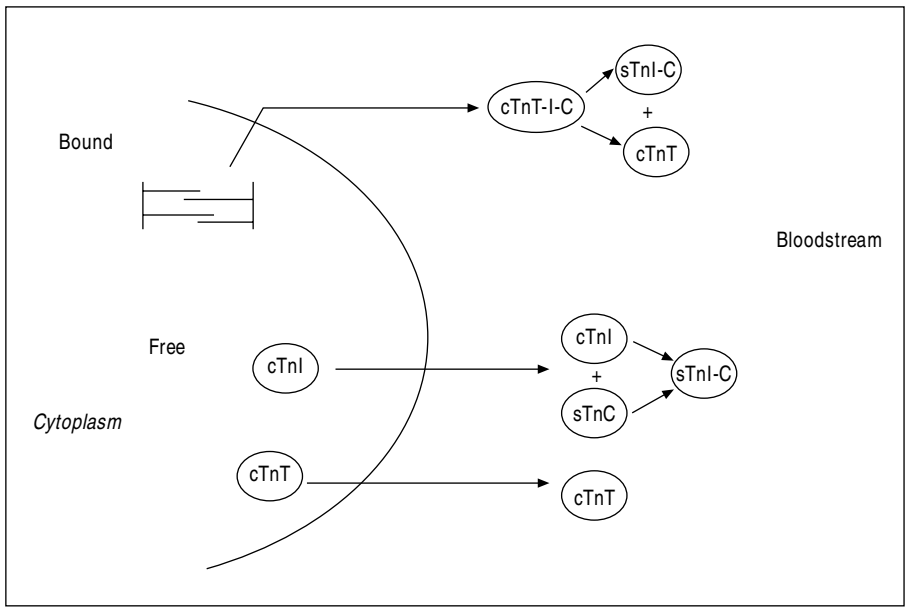

Fig. 5. Pathways for troponin release

showed some cross-reactions (4%-10%) with TnT found in skeletal muscle.[50] The cTnT test available today, in which an antibody targets the more stable part of the molecule and which can be used in both large immunoassay machines for multiple assays as well as bedside systems (point-of-care [POC]), employs a couple of antibodies which cannot detect the combined presence of cardiac isoforms that are reexpressed in striated muscle.[51] Thus, the immunoassay currently used to measure cTnT does not lead to a false diagnosis of myocardial necrosis in patients with renal failure.[52] Nevertheless, the cTnT test in current use shows that between 18% and 75% of patients with end-stage renal failure have levels above the nominal reference threshold. In this same group of patients, different ways of measuring cTnI show elevated levels in a smaller fraction of patients, from 4%-17%.[53] Long-term follow-up of patients with renal failure and detectable troponin levels has shown that elevated cTnT levels are predictive of death from cardiovascular causes for any rise in cTnT above normal, and for any rise in cTnI above the 99th percentile of a reference population. Thus, increased levels of cTnT are more predictive of future death from cardiovascular causes than are increased levels of cTnI.[54] According to these data, finding elevated levels of cTnT (and cTnI) in patients with end-stage renal failure is suggestive of myocardial damage, and that such damage carries a worse prognosis in terms of future cardiovascular events, even though causative mechanisms are still undetermined.

Sources of error in troponin tests

In addition to what has been said so far as to how antibodies used in cTnI tests vary in their affinity for the different circulating forms, other sources of error are inherent to the methods used for measuring cTnT and cTnI.

Tests for cTnT and cTnI both yield results that are lower in heparinized plasma than in blood serum; also, in earlier stages (<24 hours) of the infarct, levels obtained in heparinized plasma are lower (60%-70% of serum levels) than those seen (approximately 90%) in more advanced stages (>24 hours).[55]

The presence of excess biotin in plasma can interfere with cTnT tests. Tests for cTnI are prone to error due to various sources, such as interference by alkaline phosphatases, tricyclic antidepressants, clozapine or similar drugs, fibrin clots, hemolysis, heterophilic antibodies or rheumatoid factor. Occasional false positive results having no attributable cause have also been reported. For a more complete review of these causes of error, see Collinson et al.

Point-of-care (POC) measurement systems

The National Academy of Clinical Biochemistry[56] recommended in 1999 that institutions that were unable to turn around the results of biologic markers for myocardial necrosis in less than one hour (extraction → results) should measure such markers using POC systems.

POC testing systems make it possible to shorten the time it takes to get the results of blood components measured in emergency rooms in critical patients. Thus, they facilitate the early diagnosis of AMI and help shorten the patient's stay in the emergency room. Such systems use whole blood and share some of the features of traditional systems, except that, as a general rule, they have lower sensitivity. The need for user training and for strict quality control of tests limit their

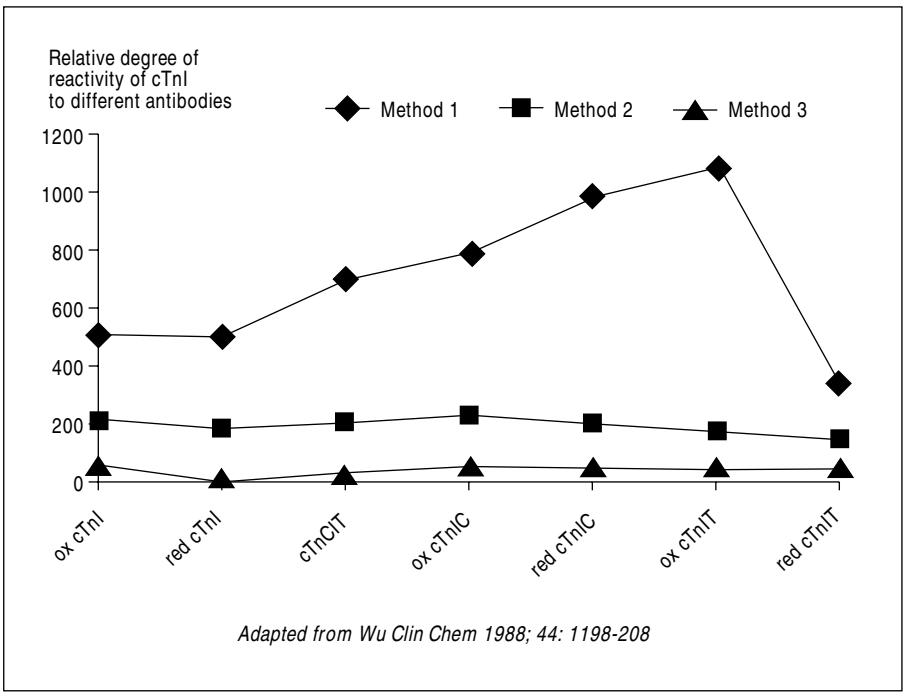

Fig. 6. Differences in the way various antitroponin I antibodies react with the molecule's circulating proteins. Modified from Wu AHB, et al.[44].

widespread use. However, in some clinical contexts they shorten the diagnostic process in a way that may be crucial to the patient's care.

In spite of the obvious advantages of these systems, their cost-efficacy ratio should be examined before they are made a part of routine care. This ratio will depend on the volume of tests performed in each laboratory and on their reliability in terms of determining which patients should be hospitalized or discharged. It has been shown that a reduction of only 2% in the number of hospital admissions offsets the excess costs of using these systems to measure troponin levels.[57]

Some POC systems can measure individual cTnT and myoglobin levels,[58] as well as combined[59] or individual levels of cTnI, myoglobin, and CK-MB, in less than 15 minutes. Their results are very much like those obtained by using conventional testing systems. For years compact desktop systems have allowed catalytic activity levels of CK and CK-MB to be measured at the bedside.

REDEFINING AMI

The improved diagnostic sensitivity and specificity of new markers for myocardial necrosis have brought about a redefinition of AMI. The early definition of AMI proposed by WHO[1,60] was quite sensitive but not very specific, since it defined as AMI only those patients showing a rise in CK or CK-MB catalytic activity were defined as AMI. Later, this definition has been arbitrarily modified by various study groups. Few pathologic processes have lacked a uniform diagnostic criterion as patently as AMI. As a result, infarction required a new definition, one that was simple and that met with the approval of the main international regulatory agencies.[61]

In 2002 several consensus documents were published, in conjunction with the American College of Cardiology (ACC), under the auspices of the European Society of Cardiology (ESC). These documents contained specific recommendations regarding the use of biologic markers for myocardial necrosis for detecting AMI.[61,62] The use of biochemical markers for redefining AMI was mostly based on the biochemical criteria provided by the National Academy of Clinical Biochemistry.[56]

The new definition of infarction was based primarily on cardiac troponin levels and, in their absence, on mass concentrations of CK-MB. A single rise in cardiac troponin above the 99th percentile of the reference population — when obtained with a method whose inaccuracy is less than 10% of this percentile — should be considered abnormal and indicative of myocardial necrosis. In a patient with myocardial ischemia, such elevations in troponin levels define the presence of AMI, even if there is no rise in CK-MB levels. Since mass concentrations of CK-MB are not entirely cardiospecific, under the new definition at least one objective measurement of CK-MB that is twice the upper reference level, or two measurements that are above this level, must be obtained if coronary ischemia is clinically present.[41,42]

Under the new guidelines for redefining AMI, a few noteworthy concepts surrounding cardiac markers are underscored:

–When coronary ischemia is clinically absent, elevated troponin levels suggest the presence of myocardial necrosis, which is not the equivalent of AMI or an ischemic process. In such cases, other causes of myocardial damage should thus be ruled out (Table 1). In a patient with myocardial ischemia, elevated troponin levels should be classified as AMI, even if CK-MB levels are normal.[62] In this regard, there has been histologic evidence of small infarcts in patients with elevated troponin and normal CK-MB levels, a fact that underscores the higher sensitivity of cardiac troponin for the diagnosis of AMI.[56,63] Around 25%-30% of patients with chest pain at rest, which is suggestive of ischemia, and who have been previously diagnosed as having UA because their CK-MB results were negative can be reclassified as cases of myocardial infarction without ST segment elevation if troponin levels are found to be abnormal.[64,65]

–Elevated cardiac troponin levels are indicative of myocardial necrosis, probably irreversible, though there is no consensus in this regard.

–In patients with myocardial necrosis, the degree to which cardiac troponin is elevated is directly related to prognosis.

–To confirm or rule out AMI, cardiac troponin measurements should include levels obtained 6-9 hours after the onset of symptoms. If no cardiac troponin level is available, the best alternative is to measure the mass concentration of CK-MB.

–Patients who have undergone angioplasty or heart surgery will probably release cardiac troponin as a result of the therapeutic procedure. In patients who have had heart surgery, no marker can unequivocally distinguish between damage resulting from a perioperative AMI and damage caused by the surgical procedure itself.

THE CLINICAL SIGNIFICANCE OF NEW BIOLOGIC MARKERS FOR MYOCARDIAL NECROSIS

In ACS with an elevated ST segment

The diagnosis of AMI is relatively reliable (>90%) in patients whose clinical symptoms are suggestive of myocardial ischemia and who have an elevated ST segment on EKG. In this group of patients, acute therapeutic decisions (fibrinolysis, angioplasty) can and should be made without delay, based solely on clinical history and the results of EKG. In such patients, all cardiac markers would point to a diagnosis of AMI, but they are not essential for making initial decisions.[24] Biologic markers will be useful not only for the retrospective diagnosis of AMI, but as a non-invasive way of assessing reperfusion (Figure 4). Because of its rapid turnover in plasma, myoglobin is the best indicator of the success or failure of reperfusion attempts; CK-MB is the marker of choice for the diagnosis of potential reinfarction, since it remains elevated in plasma less time than troponin, and for indirect assessment of the extent of myocardial necrosis (for which it is best to use the cheapest marker, such as total CK, once the diagnosis of AMI has been unequivocally established).

In ACS without ST segment elevation

Diagnostic significance

Among these patients are those who have suffered an AMI without ST segment elevation, or who have UA. It is important to be able to differentiate between these two groups of patients rapidly and effectively, since early diagnosis and treatment can improve prognosis and make for optimal use of health care resources that are normally in short supply.

Overall, the diagnostic sensitivity for AMI of all markers of myocardial necrosis after symptoms of myocardial ischemia have been present 9-12 hours is high.[66] However, currently there are no clinical parameters or diagnostic methods that will allow the diagnosis of AMI to be made during the first 9-12 hours of symptoms. Other options for reducing the time to diagnosis have been studied. They include strategies for using biologic markers and/or measuring such markers using POC systems that allow pretesting time to be shortened considerably.

Strategies based on the use of markers have included measuring the relative increase in myoglobin levels[67] or in CK-MB concentration,[68] measuring the absolute rise in CK-MB,[69] combining various markers derived from samples drawn during the first 4-6 hours after admission,[50,70,71] or using serial measurements of CK-MB concentration during the first 3-4 hours after admission.[72,73] One can conclude, based on these strategies, that most AMI patients without ST segment elevation can be diagnosed within the first 4 hours after admission.[33] The problem underlying all markers whose usefulness for making a quick diagnosis has been assessed (myoglobin, CK-MB) is their poor cardiospecificity and, consequently, their poor sensitivity for detecting small AMIs. The specificity of myoglobin within the first three hours after admission to the emergency room is less (80%) than that of CK-MB (94%).[31,32]

The availability of a cardiospecific marker like troponin has substantially changed the diagnosis of these patients. Using a cardiospecific marker increases the diagnostic sensitivity of myocardial necrosis, since the «biological background noise» made by non-cardiospecific markers (Figure 3) is avoided. On the other hand, the time required to rule out an AMI with classic markers (9-12 hours from onset) can probably

be reduced as well. As a result of troponin's improved diagnostic sensitivity and cardiospecificity, a single «positive» reading makes for the diagnosis of myocardial necrosis, without the need for further readings, which would be mandatory if the marker were less cardiospecific.[74,75] This is highly important when stratifying patients with TD and presumed ACS without an elevated ST segment, since early detection of an AMI will make it easier to apply treatments specifically designed to reduce infarct extension within a maximally efficient time and thus reduce the risk of short-term complications.[76-78]

The role of cardiac troponin in the early diagnosis of AMI has been assessed, and it has been suggested that two «negative» readings, with at least one of them obtained no less than 6 hours after onset of symptoms, makes it possible to rule out myocardial damage.[57] Other studies have shown that with the combined use of the mass concentration of CK-MB, myoglobin, and troponin on admission and 90 minutes later, one can rule out myocardial necrosis in over 95% of patients.[79,80] Recently it has been shown that serial readings of TnT from 0 to 4 hours after admission allow detection of 96.5% of patients with AMI without an elevated ST segment.[81] As a result, troponin levels are an effective tool for «ruling in» as well as «ruling out» AMI during the first few hours after onset.

Despite the acknowledged value of biologic markers for myocardial necrosis in ruling out the diagnosis of AMI, it must be emphasized that negative troponin readings and, even more so, negativity for other markers do not rule out the presence of serious coronary heart disease. In a study of patients who were consecutively admitted to a chest pain unit, significant angiographic disease (coronary stenosis above 75%) was observed in patients having cTnT levels ≥0.1 µg/L with a frequency that was significantly higher (89%) (*P*<.002) than that observed in patients having cTnT ≥0.1 µg/L (49%).[82] DeFilippi[83] has reported similar results. In both studies, the higher frequency of serious coronary disease in patients with cTnT results defined as negative is worth noting. However, in these studies, cTnT levels were classified as «positive» or «negative» before the new diagnostic guidelines for AMI were published; according to these guidelines, a significant number of patients that were considered negative for cTnT in these studies would be considered positive today.

Risk stratification

Assessing the likelihood that a patient with ACS will suffer serious cardiovascular complications (death/non-fatal AMI), either in the short or long term, is known as cardiovascular risk stratification. Risk stratification requires a multifactorial approach and is crucial when selecting the treatment and type of hospital care that the patient needs. There are numerous clinical and electrocardiographic signs and symptoms that define and stratify cardiovascular risk in these patients. Similarly, troponin levels are a powerful tool for assessing and stratifying risk.

Patients having ACS without ST segment elevation (UA or AMI without an elevated ST segment) are a very heterogeneous group with a wide range of mortality risks or of new short-term cardiac ischemic events. Consequently, the guidelines for the management of this condition that have been developed by a number of scientific entities (the American College of Cardiology, the American Heart Association, the European Society of Cardiology, the Spanish Society of Cardiology) underscore that risk stratification is one of the foremost objectives of the early work-up and treatment of these patients.[42,62,84] Initial risk stratification should be performed in the emergency room when the patient is admitted and should play a decisive role in clinical and therapeutic decisions. Good risk assessment can be carried out in the emergency room by taking clinical, electrocardiographic, and biochemical variables into account. In general, it is important to avoid oversimplifying risk stratification by applying a rigid algorithm for determining the type of treatment and hospitalization required. As mentioned earlier, estimating the short-term risk of patients is a multivariate, complex problem that cannot be explained in simple terms. An individual patient´s risk category is a continuum that can vary along the course of his/her illness and that stems from the combined assessment of all known clinical, electrocardiographic, and biochemical variables. All of these, along with the clinical judgment of an experienced physician, will determine the best treatment modality.

Assessing cardiovascular risk in ACS patients who do not have an elevated ST segment is very useful for:

–Selecting the best level of hospital care, be it an intensive care unit or a regular hospital ward, even if the patient is discharged for out-patient follow-up at a later date.[85,86]

–Identifying patients who are candidates for early revascularization and potential recipients of the strongest and most effective antithrombotic and antiplatelet agents, but who are at high risk for hemorrhagic complications and can thus generate substantial costs.[87,88]

Markers for myocardial damage play a very important role in the risk stratification of this group of patients. As noted earlier, when the ST segment is not elevated on first EKG, the diagnosis of AMI vs. UA will be made retrospectively based on biochemical markers for myocardial necrosis. The ability to detect, by measuring cardiac troponin, small necrotic areas that cannot be picked up by measuring CK-MB levels has

triggered a number of studies over the past 10 years, all of them geared toward assessing the prognostic significance of this biochemical marker. Currently no one questions the value of troponin for identifying high-risk individuals.[89,90] The value of cTnT for predicting mortality in patients[91] with ACS without an elevated ST segment is higher than that of CK-MB and cTnI, even when electrocardiographic variables are taken into account.[92,93]

All studies that have been conducted in ACS patients have shown that cardiac troponin can provide important information in terms of the short- and long-term prognosis of any serious cardiovascular complications (death/infarction/need for emergency revascularization) the patient may suffer.[39,74,94-102] In a recent metaanalysis, cTnT and cTnI levels signaled a significantly increased risk in patients who were positive for each of these markers (cTnT, relative risk [RR]=2.7; 95% confidence interval, 2.1-3.4; cTnI, RR=4.2; 95% CI, 2.7-6.4).[103] This higher risk of cardiovascular complications, together with elevated cardiac troponin levels, is independent of other risk variables, such as EKG changes and elevated markers of the inflammatory response.[104,105]

We mentioned earlier that plasma levels of a biologic marker for myocardial necrosis depend on the time that elapsed since the initial insult, on the kinetics of marker release, on the speed with which the marker is cleared from the plasma, and, most importantly, on the measurement method employed, particularly its sensitivity. For all these reasons, the first determination of a necrosis marker can turn out to be negative in patients who will eventually have positive results. In such patients it is all right to measure these markers serially. In a study of 734 patients with ACS which looked at mortality among this group of patients, cTnT levels at the time of admission and 8 hours later provided more valuable prognostic information while the patient was in the hospital and 30 days later than a single reading on admission. Subsequent measurements did not yield additional prognostic information.[106] Consequently, measuring troponin once when the patient is first admitted to the emergency room and at least once more over the next 8-12 hours would seem advisable.[107]

It must be emphasized that patients whose troponin results are negative are not always low-risk. Lindhal reported a 5% incidence of death or non-fatal AMI after 5 months in this type of patient,[108] and Galvani found a 5% incidence of death or non-fatal AMI after 30 days in patients with Braunwald's class III UA.[100] Patients whose troponin test results are negative can present with serious coronary heart disease and a high risk of recurrent ischemia requiring coronary revascularization.[98,101,109,110] Once again, however, defining a troponin test result as «positive» or «negative» should be done in accordance with the new recommendations, and data obtained before such recommendations were issued should be assessed with caution. In some studies cardiac troponin levels obtained by a method whose inaccuracy exceeds 10%, the recommended limit, have been considered «positive»; likewise, in some cases levels have been considered «negative» that would be considered «positive» by today's definitions.

Therapeutic guidelines

Among patients with ACS that do not have an elevated ST segment, increased cardiac troponin levels have been used to identify, both retrospectively as well as prospectively, individuals who can benefit from strong antithrombotic therapy, such as low-molecular-weight heparins[111-113] and platelet glycoprotein IIb/IIIa receptor antagonists.[114-115] For instance, in the PRISM (Platelet Receptor Inhibition in Ischemic Syndrome Management) study, treatment with tirofiban was associated with a relative decrease of nearly 70% in deaths/AMI after 30 days in patients with cTnT or cTnI levels that were defined as high in the study, whereas there was no benefit for patients whose troponin levels were not elevated. A number of studies have shown that treatment with platelet IIb/IIIa receptor antagonists reduces deaths or infarcts by 40%-70% in ACS patients without an elevated ST segment and with high basal troponin levels.[116-120] This benefit is greatest for patients who undergo invasive treatment (angioplasty) early on.

The risk ratio for reduced deaths or non-fatal AMI seen in the group of studies showing the benefits of treatment with IIb/IIIa glycoprotein inhibitors in the subgroup of patients with ACS who have no elevation of the ST segment and who are positive for cTnT is very strong: 0.34, with a 95% CI between 0.19 and 0.58. The results of these studies contradict the results obtained in the study on the Global Use of Strategies to Open Occluded Arteries-IV Acute Coronary Syndromes,[121] in which the use of abciximab was of no benefit in a population of ACS patients in which a single positive cTnT result (defined as ≥0.1 μg/L) was one of the criteria for treatment with abciximab. The unexpected results of the GUSTO IV ACS study can be explained by factors such as differences in inclusion criteria among studies as well as differences in the troponin tests performed at participating centers and at a central laboratory. This circumstance, which is seen in many multicentric studies, is a factor that increases the imprecision and inaccuracy of troponin determinations.

According to a recent metaanalysis of the main randomized trials using IIb/IIIa platelet receptor antagonists that comprised 11 059 patients for whom baseline troponin levels were available, treatment with these agents produced a 15% reduction in the risk ratio of death or non-fatal infarct in those patients whose troponin results were positive on admission, relative to

patients who did not receive this treatment.[122] These results would support the use of troponin for identifying ACS patients without an elevated ST segment who would benefit from treatment with powerful antiplatelet aggregation agents.

More recently, the TACTICS study[123,124] showed the usefulness of measuring cTnT or cTnI on admission so as to optimize the treatment strategy in this type of patient. In this trial, the benefit of giving patients glycoprotein IIb/IIIa receptor inhibitors, followed by an early invasive intervention, is almost exclusively limited to patients having «positive» troponin results. Such findings are consistent with a study in a subgroup of patients of the FRISC II study (Fragmin and Fast Revaascularization during Instability in Coronary artery disease). This substudy showed decreased mortality at one-year follow-up in patients with baseline cTnT levels above 0.1 µg/L who had undergone some form of invasive therapy early on.[125]

Even though other clinical predictive factors, such as ST segment depression, are also useful for identifying patients who are likely to benefit from early invasive therapy,[121] cardiac troponins provide information in a greater number of patients. Specifically, they identify more patients (60% for cTnI and 54% for cTnT levels, vs. 38% for ST segment depression) who would benefit from invasive therapy rather than conservative treatment. Measuring this biologic marker should thus be made a part of risk stratification in patients who are candidates for such therapy.[122] Again, given the economic costs and risks associated with these interventions, the role of troponin in identifying patients who can benefit from them is extremely important.

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