

La Lettre

CARDIAC MARKERS

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Currently, numerous markers of cardiac distress enable rapid diagnosis and, in the majority of cases, enable the lesion to be dated.

There are two categories of marker:

- markers of an acute event (myocardial infarction (MI) and angina pectoris)
- markers of a chronic condition (ventricular dysfunction and heart failure)

INTRODUCTION

A 'good' cardiac marker is a biological parameter that can be determined in blood and has the following characteristics:

■ **High specificity:**

The marker must be present exclusively in myocardial tissue and must be present in very low quantities in, or even absent from, the biological media of healthy subjects.

■ **High sensitivity:**

The marker must be present at a significant level that is sufficient to enable measurement in the tissues post-lesion. The release from the myocardium into the blood stream is to be fast so that the marker can be used as an early diagnostic tool. However release must continue for a sufficient period to enable orientation of late diagnosis.

In addition, the marker is required to have certain analytical characteristics:

- easy measurement using a fast method,

- reliable and inexpensive,

- easy to implement, sometimes even at the patient's bedside.

The processes used are to enable a result to be rapidly obtained with sufficient precision and accuracy to diagnose or rule out a disease.

Lastly, the cardiac marker is required to have clinical characteristics enabling it to be used for diagnosis but also conferring therapeutic and prognostic criteria on the marker.

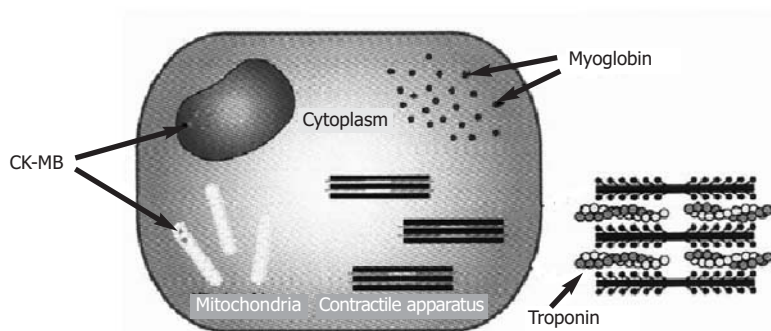
MARKERS OF ACUTE EVENTS

There are three main markers of acute events:

- myoglobin
- CK-MB
- troponin

The size of those markers and their subcellular location explain the kinetics of their appearance in biological media.

HEART MUSCLE CELL



The size and sub-cellular distribution of the protein or enzyme determines the rate of appearance of the biomarker in the systemic circulation.

Myoglobin

Myoglobin is a small protein located in the cytoplasm of all smooth, cardiac and striated muscle cells. It consists of the heme protein that binds oxygen with a greater affinity than that of haemoglobin.

Myoglobin plays an essential role in the transport of oxygen to mitochondria enabling adenosine triphosphate (ATP) production and muscle work.

The catabolism of myoglobin mainly occurs at intramuscular level under the action of proteolytic enzymes. The metabolites are eliminated in the urine.

Myoglobin is present in all muscle cells and is therefore not specific to the myocardium. Normal muscle turnover is such that myoglobin can always be detected in serum at levels that depend on the methods used.

In the urine, myoglobin concentrations are extremely low: from 0 to 4 mg/L, and frequently below the limit of detection of the method used. Moreover, in that medium, myoglobin, which is not surrounded by proteins, is very unstable. Thus, urinary assay, in order to be reliable, must be conducted 3 hours post-sampling.

However, when correctly conducted, the test may be important with regard to determining the risk of acute kidney failure, an important complication of massive myoglobinuria.

Myoglobin levels increase in all settings of muscle stress (intense exertion, toxic or non-toxic muscular lesions, hereditary degeneration, etc.). However, the small size of the myoglobin molecule and its availability within the cell result in rapid release into the blood stream post-lesion. Myoglobin thus constitutes the earliest parameter for diagnosis of myocardial infarction.

At the cutoff of 70 ng/mL, the nephelometric method of determining myoglobin has the following characteristics: 83.3% sensitivity; 95.5% specificity; 93% positive predictive value; 88.7% negative predictive value.

CK-MB fraction

Creatine kinase (CK, formerly creatine phosphokinase (CPK)) is an enzyme found in two intracellular sites (cytosol and mitochondria) that reversibly catalyzes the phosphorylation of creatine by ATP.

CK is a dimer consisting of two subunits with independent catalytic activity.

The subunits are of two types: M (for muscle) and B (for brain). The combination of those subtypes yields the three CK isoenzymes: CK-BB (CK-1); CK-MB (CK-2) and CK-MM (CK-3) whose relative proportions in the cell depend on the tissue in which the cell is located. The brain only contains isoform BB. The latter is also strongly present in the organs of the gastrointestinal tract and in parenchymatous organs (uterus, prostate, kidneys, liver, etc.).

The highest proportion of MB is found in the heart.

In the serum of healthy subjects, only the predominant MM form can be detected due to the mass of the skeletal muscles (100-fold greater than the mass of cardiac muscle).

In consequence, the MB fraction is of value as a cardiac marker. Immunoinhibition methods were long used to detect the MB fraction but are now becoming increasingly neglected. Those methods are subject to interference by the presence in serum of:

- isoenzymes BB which induce an overestimate
- macro-CK, agglutination of the enzyme around immunoglobulins, resulting in such surprising results as a CK-MB concentration greater than the total CK concentration due to the absence of immunoinhibition.

The new mass-based immunometric methods are currently preferred. Those methods enable CK-MB assay by weight and generally use two monoclonal antibodies.

Since CK-MB is located in the cell nucleus and mitochondria, its release in the event of a lesion is slower than that of myoglobin. In addition, before CK-MB is released into plasma, it is released into lymph. This explains why the maximum release occurs 4 to 8 hours post-lesion.

Elevation of CK-MB is considered characteristic of myocardial infarction. However, elevated levels are also observed in the context of severe muscular disorders, intense exertion and in patients on drugs such as statins.

Troponin

Troponin is a microfibrillar protein of the muscle contraction system with a relative mass of about 80 kDa. Troponin consists of 3 subunits coded for by different genes: troponins T (TnT), C (TnC) and I (TnI).

■ TnT

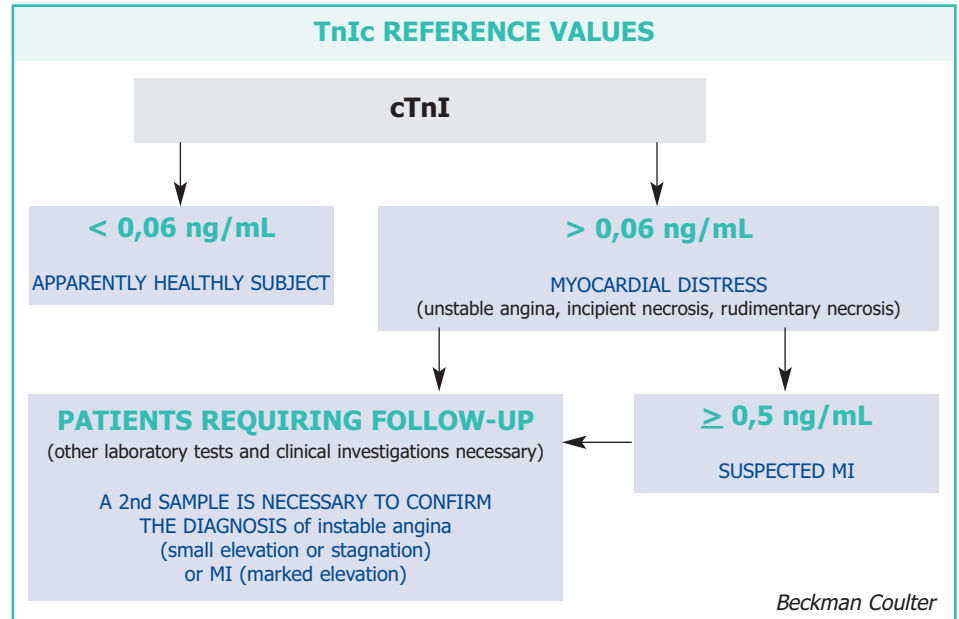
TnT consist of two distinct isoforms with specific tissue locations: striated muscle or myocardium (cTnT). The cellular distribution of cTnT - 6% in the plasma; 94% in the myofibrils - results, in the event myocardial necrosis, in two-phase release of cTnT (release from the cytoplasm pool precedes release from the myofibril pool).

■ TnC

TnC is responsible for the calcium binding necessary for muscle contraction.

■ TnI

TnI is the regulating subunit of troponin involved in the control of muscle contraction. TnI regulates the ATPase activity of the actin/myosin complexes of striated muscle fibre. Three tissue-specific isoforms of TnI have been identified, of which cTnI in the myocardium. The cardiac isoform contains an additional 30 amino acids in the N-terminal portion of the molecule, increasing the relative mass (22.5 kDa) and conferring a distinct immunological specificity which currently constitutes the basis for assay of the molecule.



Diagnostic value

In addition to enabling diagnosis of true myocardial infarction (MI), troponin also enables detection of myocardial distress in which the necrosis is less marked such as in unstable angina.

Any troponin elevation is in fact to be considered a sign of myocardial distress and to be taken into account.

The patient must be monitoring to investigate for any underlying diseases.

The usual values for the parameter are of two types: the cutoff characterizing infarction is determined by generating the receiver operating characteristic (ROC) curve and the reference cutoff (97.5 percentile), is used to characterize myocardial distress.

Value in clinical practice and treatment follow-up

■ Reperfusion follow-up

A washout phenomenon consisting in interstitial flushing caused by restored circulation is observed. This results in a massive and early release of the substances that accumulated in the interstitial compartment during ischemia.

A rapid falloff in the parameter follows, reflecting the efficacy of reperfusion. Two assays on specimens drawn at an interval of 90 minutes are recommended.

■ Heart surgery follow-up

Troponin elevation has a predictive value with respect to the occurrence of a complication due to myocardial infarction.

■ Heart transplant follow-up

Troponin may be considered a more sensitive marker of rejection than biopsy specimen histology.

The diagnosis of myocardial infarction is the subject of recommendations formulated by the French and international cardiology societies:

The use of two markers is recommended:

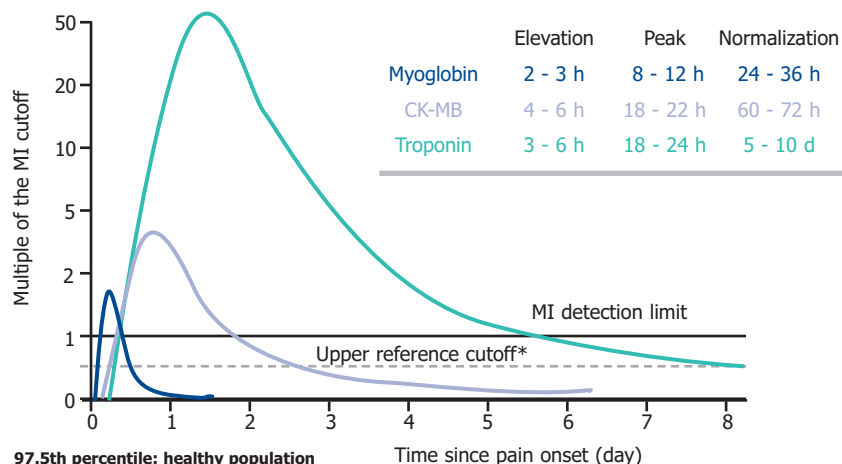
- early: myoglobin
- later: troponin

Total CK is no longer recommended and use of lactate dehydrogenase (LDH) and aspartate amino transferase (ASAT) is to be discontinued.

The laboratory criteria for ruling out necrosis are based on the serial determination of the parameters: on admission (T0), T0+6h, T0+12h, and, in the US recommendations, T0+24h.

KINETICS OF ACUTE LESION MARKERS

after NACB 1999



Kinetics of acute lesion markers

Serial determination is based on the kinetics of the markers and justified by the need to determine the patient's position on the curves.

Myoglobin, the first marker released, rises rapidly over 2 to 3 hours, peaking at about 8 hours post-lesion. In contrast, the fall to baseline is fast and in 24 hours the patient's myoglobin levels returns to normal. For that reason, the parameter must be measured at admission but also at hours 4 and 8.

CK-MB, due to its mitochondrial site, is released into the blood stream 4 to 6 hours post-lesion. CK-MB peaks in about 20 hours and returns to baseline in 72 hours.

The initial kinetics of troponin are the same as those of CK-MB but troponin returns to baseline in 5 to 10 days. Troponin is thus of additional value in retrospective diagnosis. The cardiac etiology of a pain may thus still be determined two days post-onset.

Serial assays: on admission and at least at hours 4, 8 and 24, enable the prognostic characteristics of the parameter to be exploited.

Any break in falloff kinetics is a poor prognostic factor for disease progression.

CHRONIC DISEASE MARKERS

Natriuretic peptides: BNP

Three natriuretic peptides regulate the water-sodium balance by increasing diuresis, natriuresis and glomerular filtration and decreasing sodium retention.

All 3 peptides have a ring structure closed by a disulfide bridge. They differ biochemically in terms of the terminal chain.

- ANF: Atrial Natriuretic Factor
- BNP: Brain Natriuretic Peptide
- CNP: Type C Natriuretic Peptide

The synthesis of those peptides and the type of stimulation to which they are subject differ, enabling their differential use in the diagnosis or follow-up of heart disease.

Peptide	Origin	Stimulus
ANF	Atrium	Atrial distension
BNP	Ventricle	Ventricular overpressure
CNP	Endothelium	Endothelial stress

CNP is located at endothelial level and at the present time no method of determining blood CNP is available.

Due to its almost exclusive ventricular location, BNP appears to be of greater value and to be more sensitive in the diagnosis and follow-up of heart failure.

BNP is mainly secreted by the left ventricle in response to stimuli, particularly mechanical stimuli such as ventricular distension due to volume expansion or a pressure increase.

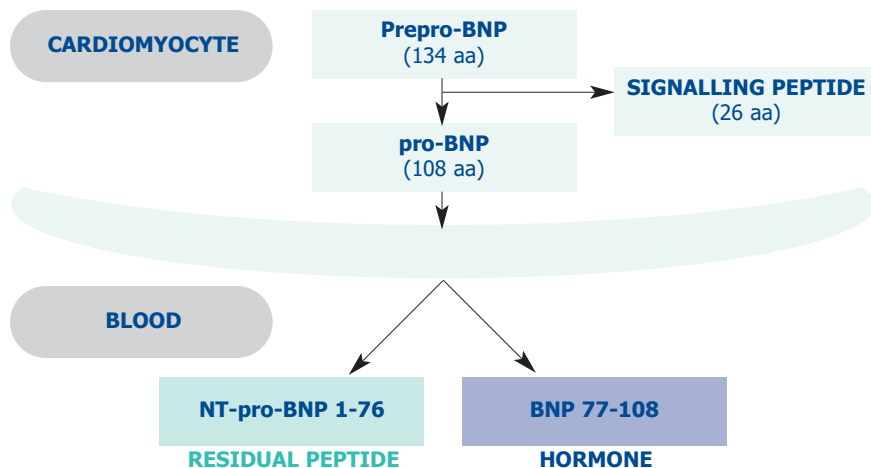
Dilatation of the ventricle thus induces an increase in BNP which can be identified in blood samples.

BNP is synthesized in the cardiocytes. Pro-BNP yields two circulating metabolites secreted in equimolar quantities:

- NT-pro-BNP: an inactive molecule which is in fact a residual peptide
- BNP: the peptide with hormonal activity

The above two metabolites can be used in disease diagnosis or patient follow-up. Antibodies are available for each. Both would appear to have the same diagnostic and prognostic characteristics and the same value with respect to follow-up treatment.

BNP SYNTHESIS, SECRETION and ELIMINATION



J. Mair, Clin Chem Lab Med ; 39/7 : 571-588

However, the antibodies were developed by different manufacturers. The assay is conducted by immunoenzymatic and immunoradiometric methods based on monoclonal antibodies.

Both peptides are excreted by the renal route.

The values obtained in settings of kidney failure have long been debated for both peptides.

It has now been shown that both BNP and NT-pro-BNP increase in the context of kidney failure:

- due to the increase in blood volume, defective renal elimination and decreased cardiac functions for BNP ;

- due to decreased glomerular filtration for NT-pro-BNP.

The latter is slightly more influenced by the exacerbation of renal function than BNP. The difference may be due to a difference in the clearances of the two peptides.

However, the variation in those two parameters remains strongly significant in the event of dyspnoea of cardiac origin, even in the presence of kidney failure. The two parameters should therefore be determined in that context.

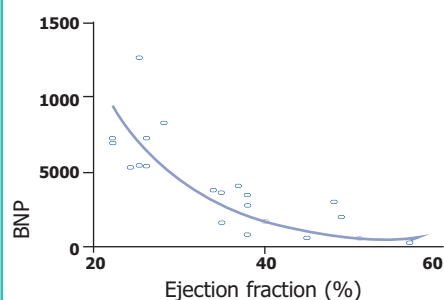
Diagnostic value: heart failure

Heart failure is a major public health issue. Its prevalence in Europe is of the order of 0.4 to 2%, without including asymptomatic patients.

Incidence increases rapidly with age (mean: 74 years) and the ageing of the population explains this increase.

In the absence of adequate treatment and a curable cause, the mean 4-year mortality rate is 50%. Dyspnoea is a major clinical sign of acute heart failure but may also be due to pulmonary disease, infection or embolism. A marker for differential diagnosis is therefore of value.

BNP CORRELATION with EJECTION FRACTION



Bevilacqua et al., Clin. Chem. 1997 ; 43 : 2439-40

In 1997, Belavicqua et al. demonstrated an inverse correlation between BNP level and cardiac ejection fraction. The lower the latter, the higher the BNP, which may reach levels of up to 30-fold the baseline value.

Thus, the 100 ng/L cutoff for BNP has 93% sensitivity and 79% specificity with regard to detecting patients with an ejection fraction of less than 50%. In the DAO Q study, BNP was determined in patients presenting to an emergency department with dyspnoea. When the dyspnoea was due to pulmonary disease, the mean BNP level was about 90 ng/L. In the event of congestive heart failure, the level may reach 1000 ng/L.

Outside of the settings of acute dyspnoea, BNP is also an excellent marker of left ventricular dysfunction. A normal BNP level has a 97% negative predictive value for the diagnosis of systolic left ventricular dysfunction. However, the parameter cannot be used to differentiate between systolic and diastolic dysfunction. Nonetheless, it remains proportional to the magnitude of the failure.

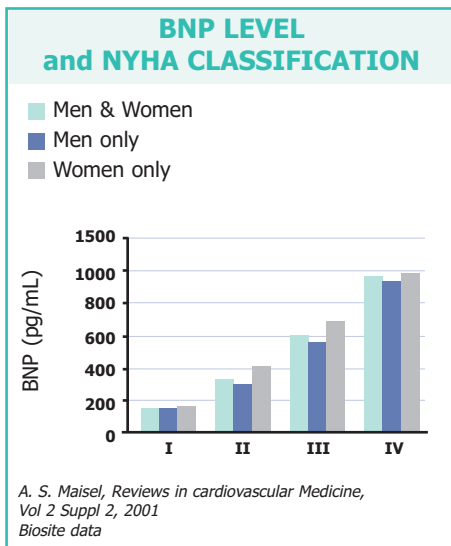
The clinical signs of heart failure are dyspnoea, asthenia and oedema.

The New York Heart Association (NYHA) has formulated an international classification of heart failure:

- **Class I:** no limitation of physical activity; ordinary physical activity does not cause undue fatigue
- **Class II:** slight limitation of physical activity; ordinary physical activity results in fatigue, palpitations or dyspnoea
- **Class III:** marked limitation of physical activity; less than ordinary activity causes fatigue, palpitations or dyspnoea
- **Class IV:** unable to carry out any physical activity without discomfort; symptoms of cardiac insufficiency at rest.

Patient assessment by a 6-minute treadmill test shows a good correlation between the severity of heart failure and the number of meters covered over the 6 minutes.

BNP is highly correlated with functional impairment. The shorter the distance covered by the patient, the higher the levels of plasma BNP.



Thus, in patients presenting to an emergency department with dyspnoea, the BNP cutoff of 100 pg/mL makes it possible to distinguish the patients primarily presenting with heart failure (elevated BNP) from those without heart disease. The main value thus resides in the negative predictive value of the parameter.

Between 100 and 400 ng/L: Transthoracic echocardiography is necessary to investigate for heart disease or eliminate an alternative diagnosis (acute cor pulmonale, pulmonary hypertension or pericardial effusion).

Above 400 ng/L: The diagnosis of acute heart failure is highly probable.

Value in follow-up treatment

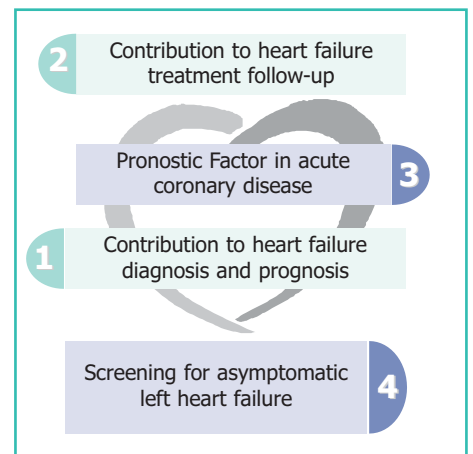
During the ambulatory follow-up of patients effectively treated for heart failure, the BNP values are greater than the normal range but stable (200 to 300 ng/L). Their time course can thus be used to orient treatment. BNP enables good and poor responders to treatment to be distinguished. As a criterion, BNP is reported to be superior to clinical course. Any increase over baseline is considered a sign of disease exacerbation.

In addition, BNP is providing new openings for the treatment of heart failure either directly using recombinant BNP or indirectly using BNP-catabolism inhibitors. However, the BNP assay kits do not enable endogenous BNP to be distinguished from exogenous BNP. In that case, only pro-BNP enables effective patient follow-up.

Pronostic value

BNP levels have been shown to increase late in the course of myocardial infarction. BNP therefore does not constitute a diagnostic marker but rather a good marker of cardiac remodelling.

In addition, BNP constitutes an independent indicator of post-infarction survival.



BNP is correlated with the risk of serious events at 30 days and 10 months.

In that setting, BNP increases after 24 h, then slowly falls off toward the baseline (4 to 5 weeks). A second peak may occur after 5 to 6 days. The latter resurgence is reported to be a poor prognostic factor with respect to the disease course. The resurgence is greater in patients experiencing recurrence and those presenting with a decreased ejection fraction or a persistently high CK-MB level.

BNP may thus prove particularly valuable for the identification of patients for whom an invasive strategy would be the most beneficial.

BNP and pro-BNP are the only laboratory markers of heart failure. Their major interest resides in their negative predictive value, while they are also well correlated with disease severity.

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