



**ASSOCIATION OF CLINICAL  
BIOCHEMISTS IN IRELAND**

**Guidelines on the Use of Biochemical  
Cardiac Markers and Risk Factors**

**Seán Cunningham**

**Peadar McGing**

**Tony McGill**

**Helen Grimes**

**Alan Balfe**

**Nuala McCarroll**

Editorial Committee:  
Peter Brown, Martin Healy, Alan Balfe, and Anne Killalea

## ***PREFACE***

*These Guidelines, the third in a series produced by the Association of Clinical Biochemists in Ireland (ACBI) on different aspects of the Clinical Biochemistry Service, have been developed and produced on behalf of the Scientific Committee of the Association to promote the most appropriate and cost-effective use of cardiac markers in the diagnosis, risk assessment and management of patients. The guidelines should prove useful, not only to the laboratories carrying out these assays, but also to General Practitioners and non-specialist medical and nursing staff.*

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*Niamh Cavanagh FRCPath Eur Clin Chem  
Chairman, ACBI*

*Martin Healy PhD  
Chairman,  
ACBI Scientific Committee*

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## **GUIDELINES ON THE USE OF BIOCHEMICAL MARKERS IN ACUTE CORONARY SYNDROMES**

### **Introduction**

Rapid developments in biochemical markers of myocardial ischaemia and in treatment regimes have revolutionised the approach to diagnosis and management of acute coronary syndromes in recent years. Using classical diagnostic regimes, many patients are admitted to CCU who might more properly have been discharged or admitted to less intensive wards. Furthermore, it has been estimated that in the US between 2 and 8% of patients presenting with chest pain are discharged, but go on to have a myocardial infarction (MI).

### **W.H.O. Classification of Myocardial Infarction**

Under the classical W.H.O. definition of myocardial infarction, which is more than 20 years old, an MI was diagnosed when any two of the following were present:

1. Chest pain with a cardiac pattern
2. New ischaemic changes in ECG such as ST segment elevation, ST depression, T-wave changes
3. Elevation and subsequent fall of cardiac enzymes (creatine kinase, CK-MB, LDH and AST) to at least twice the upper limit of normal over a typical time course.

There are a number of difficulties with these diagnostic parameters. Cardiac chest pain must first be differentiated from numerous other causes of chest pain such as pericarditis, pleurisy, pulmonary embolism, oesophagitis, aortic aneurysm, ulcers, and gall bladder disease. While pain can be very severe in some patients, in others it may be mistaken for indigestion and indeed may be silent in 20% of patients, particularly the elderly and those with diabetes mellitus. The ECG may be normal or equivocal in 20-30% of patients and may take some hours to evolve. Cardiac enzyme tests lack specificity and early sensitivity.

## **Myocardial Infarction Redefined**

The new and still evolving approach to MI has been encapsulated in a consensus document from the European Society of Cardiology / American College of Cardiology (Journal of the American College of Cardiology 2000, 36: 959-69). The new definition of MI is:

Typical rise and fall of biochemical markers (Cardiac Troponin, CK-MB), with at least one of the following:

- a) Ischaemic symptoms
- b) Pathological Q-waves on ECG
- c) ECG changes indicative of ischaemia e.g. ST elevation or depression
- d) Following coronary artery intervention (e.g. PTCA)

There is a growing perception that unstable angina and non Q-wave MI are part of a continuum. In this new approach, the importance of measurement of the new protein markers such as Troponin and CK-MB by immunoassay has become central.

In the next section, classical and newer biochemical markers are discussed under the headings physiology, time course of release, analytical aspects, diagnostic and prognostic use, and clinical sensitivity and specificity. An ideal marker of myocardial damage would:

- a) be found only in the myocardium and not in other tissues, either normally or pathologically
- b) be released rapidly following damage
- c) persist long enough in the circulation
- d) be released in amounts proportional to damage
- e) be easily measurable (accurate, precise, inexpensive).

In general the available biochemical markers do not yet meet all these criteria.

## **PART I**

### **BIOCHEMICAL MARKERS IN ACUTE CORONARY SYNDROMES**

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### **Aspartate Transaminase (AST)**

One of the oldest markers of MI, AST (together with CK and LDH) is still in use in some laboratories. However it has little value as a marker of cardiac dysfunction and has been superseded by newer markers.

### **Lactate Dehydrogenase (LDH)**

Another classical marker, LDH too has been superseded by the newer markers and should not be included in the 'cardiac profile'.

The only place where LDH might be used as a marker of cardiac dysfunction is where a troponin assay is not available and the chest pain under investigation occurred more than 24 hours previously.

## **Total Creatine Kinase (CK)**

### **Physiology**

CK catalyses the reversible dephosphorylation of creatine phosphate to creatine and ATP. This is the faster of the body's two mechanisms for regenerating ATP following ATP-consuming processes such as muscle contraction.

### **Time Course of Release**

Total CK starts to rise at 3 to 6 hours post onset of chest pain. It peaks between 12 and 24 hours, usually with or slightly later than CK-MB. Concentrations return to normal after 2 to 3 days (but take longer where peak CK is extremely high).

In a patient with MI presenting early there will be a characteristic rise and fall in CK. Late presenting patients may only show the fall.

Broadly speaking the greater the cardiac damage the higher the peak and the longer it takes to return to normal.

### **Analytical Factors**

CK is measured in plasma or serum, usually at 37°C.

Haemolysis interferes with some CK assays.

### **Diagnostic Use – Clinical Sensitivity and Specificity**

Timing of cardiac marker sampling is critical.

CK is neither sensitive nor specific at less than 6 hours post MI.

Acute MI (AMI) should not be ruled out on the basis of a single CK or MB less than twelve hours post onset of chest pain.

Sensitivity of Total CK for AMI approaches 95% after 12 hours.

Some individuals may have low baseline CK. A significant rise in CK may still be within the reference range.

Specificity of Total CK for AMI is low and a more cardiac-specific test is required as second-line where CK is used as first-line.

CK Slope: The change in CK per unit time has been used in the past. A rise of about 3.5% has been employed as an indicator of MI. This test has not been universally accepted.

### **Prognostic Use**

CK is not a useful prognostic marker. Though peak plasma CK level does correlate well with infarct size, other factors, such as location of infarct, are more important for clinical outcome.

In patients on lipid lowering treatment, statins (HMG-CoA reductase inhibitors) can cause a myopathy and some CK monitoring may be needed.



**Reference Ranges**

Ranges vary from lab to lab but are approximately as follows for a Caucasian population, when assayed at 37°C

Males 30 – 180 U/L (0.50 – 3.00  $\mu$ kat/L)

Females 30 – 140 U/L (0.50 – 2.33  $\mu$ kat/L)

Neonates: 2-3 x adult values.

Children > 6 weeks: adult values.

Afro-Caribbeans (particularly males) have CK levels up to twice the levels of Caucasians. Asians and Hispanics have intermediate values.

## **CKMB – Activity Assay**

### **Physiology**

CK in plasma exists in 3 forms: MM (approx 97%), MB (approx 2%), and BB (trace). CKMB is primarily of cardiac origin.

### **Time Course of Release**

MB usually starts to rise at 2-4 hrs. It peaks at 12 to 24 hours (usually 12-18 hours) and returns to normal within 72 hours.

CKMB half-life is shorter than that of CKMM, therefore its rate of decline (post peak) is greater than for total CK.

Timing of cardiac marker sampling is critical. AMI should not be ruled out on the basis of a single CKMB less than twelve hours post onset of chest pain.

### **Analytical Factors**

Activity assays are immunoinhibition type. Antibodies are used to block CK-M activity and the remaining activity is presumed to be the CK-B part of CK-MB; this measured activity is multiplied by 2 to give the MB result. This assay is relatively inexpensive but is subject to interference from CK-BB (from tumours or damage to any tissue other than cardiac or skeletal muscle) and macro CKs (primarily immunoglobulin bound CK-BB and oligomeric forms of mitochondrial CK).

MB immunoinhibition assays should be run with blank subtraction (background averages 5-10 IU/L). In practice this is not usually done.

CK-MB activity greater than 20% of total CK should be queried as possible method interference and further investigation using MB mass or a Troponin is advised.

Any haemolysis interferes with activity assays.

### **Diagnostic Use – Clinical Sensitivity and Specificity**

MI diagnosis by CKMB depends on having both MB activity and fraction (% of total CK) raised, since if serum total CK is elevated due to skeletal muscle damage CKMB values will be above the reference limit.

When sampled appropriately MB activity can give over 95% sensitivity and specificity.

True CKMB may be increased following severe physical exercise.

High CKMB fraction is associated with regenerative and type I fiber myopathies. It has also been reported in tumours (rare) and hypothyroidism.

Very young children may have increased proportion of the B subunit (as MB and/or BB).

**Concomitant skeletal muscle damage**

Detection of cardiac muscle damage in the presence of concomitant skeletal muscle damage is difficult. Even if the patient has had an MI the small amount of MB in the serum relative to the level of MM from skeletal muscle damage dilutes the MB fraction. Because of the likelihood of a false negative result the MB fraction should not be reported with very high CK results (e.g. if query MI in rhabdomyolysis patient)

**Prognostic Use**

No prognostic value.

**Reference Ranges**

Ranges vary from lab to lab but are approximately as follows for a Caucasian population, assaying at 37°C

MB activity: 0 - 15 U/L (0 - 0.25  $\mu$ kat/L)

MB fraction: 0 - 5 %;

For diagnosis of MI it is considered desirable that both activity and fraction are elevated.

## **CKMB – Mass Assay**

### **Physiology**

CK in plasma exists in 3 forms; MM (approx 97%), MB (approx 2%), and BB (trace). CKMB is primarily of cardiac origin.

### **Time Course of Release**

MB usually starts to rise at 2-4 hrs. It peaks at 12 to 24 hours (usually 12-18hrs) and returns to normal within 72 hours.

CKMB half-life is shorter than that of CKMM therefore its rate of decline (post peak) is greater than for total CK.

Timing of cardiac marker sampling is critical. AMI should not be ruled out on the basis of a single CKMB less than twelve hours post onset of chest pain.

### **Analytical Factors**

Mass assays are immunometric type. Monoclonal antibodies are directed against the MB subunits. Generally the presence of CKBB or CKMM do not interfere with the assay. All immunoassays may be susceptible to false positive or false negative results due to interfering heterophilic antibodies.

Haemolysis: interference is only seen with severe haemolysis.

### **Diagnostic Use – Clinical Sensitivity and Specificity**

CKMB mass improves clinical sensitivity and considerably improves clinical specificity compared to Total CK. CKMB mass gives improved sensitivity versus MB activity, especially earlier sensitivity.

When sampled appropriately MB mass can give over 98% sensitivity and specificity.

If serum total CK is elevated due to skeletal muscle damage, CKMB levels will be above the reference limit. Traditionally MI diagnosis by cardiac enzymes has depended on having both MB activity and fraction (% of total CK) raised. Though some workers have expressed doubts about use of Relative Index of MB mass ( $[\text{MB in } \mu\text{g/L} \times 100] / \text{Total CK in U/L}$ ) it enables one take some account of MB fraction. In general use of 'MB fraction' increases specificity but decreases sensitivity.

True CKMB may be increased following severe physical exercise.

High CKMB fraction (activity or mass) is associated with regenerative and type I fiber myopathies. It has also been reported in tumours (rare) and hypothyroidism.

**Concomitant skeletal muscle damage**

Detection of cardiac muscle damage in the presence of concomitant skeletal muscle damage is difficult. Even if the patient has had an MI the small amount of MB in the serum relative to the level of MM from skeletal muscle damage dilutes the MB fraction. Because of the likelihood of false negative result MB fraction should not be reported with very high CK results (e.g. if query MI in rhabdomyolysis patient).

**Prognostic Use**

No prognostic value.

**Reference Ranges**

Ranges vary from lab to lab but are approximately as follows, assaying at 37°C

CKMB mass: 0 - 7 µg/L.

RIM (Relative Index of MB Mass): As a rough guide RIM of less than 2“%” suggests that an elevated CK with elevated MB mass is probably not of cardiac origin. Elevated MB mass with RIM over 5“%” of (elevated) CK supports AMI but clearly is not diagnostic on its own.

## **Cardiac Troponin I**

### **Physiology**

The Troponin complex, located on the myosin filament of the actinomyosin contractile apparatus of muscle, consists of:

Troponin T, which binds the Troponin complex to the tropomyosin strand. Troponin C, which binds calcium and regulates contraction.

Troponin I (TnI) which inhibits myosin ATPase activity.

There is one cardiac specific isoform of TnI, with a molecular weight of 26.5kDa.

### **Time Course of Release**

TnI becomes elevated 4 – 9 hours following an MI and remains elevated for about 5 days. Concentrations can increase 10 – 1000 times the upper limit of values found in normal healthy subjects. Smaller increases may be found in patients presenting with unstable angina, sepsis, pericarditis, injury due to trauma etc.

### **Analytical Factors**

The commercially available assays for TnI have very good analytical specificity. There is as yet little standardisation between the various TnI assays resulting in wide variation in troponin ranges. TnI is released into the circulation in various forms: free TnI, TnI complexed with one or more Troponin components, or troponin which has been partially degraded and chemically altered. While all forms increase following an MI, assays from different manufacturers detect these components in varying degrees. The stability of TnI in blood specimens also varies depending on the assay chosen. Heparin and fibrinogen affect some assays and therefore serum and plasma are generally not interchangeable.

### **Diagnostic use - Clinical Sensitivity and Specificity**

#### **Myocardial infarction**

The clinical sensitivity of TnI for acute myocardial infarction is 90 – 100% at 12 – 24 hours following the event. Estimates of the early sensitivity of TnI vary:

49% sensitivity 0-4 hrs after onset of chest pain, similar to TnT and CKMB mass (Mair et al)

63% sensitivity on presentation and 98% sensitivity 4 hrs after presentation, clinical specificity >90% (Heeschen et al)

> 90% sensitivity and 95% specificity 8 hrs post onset of chest pain (Ebell et al)

#### **Unstable angina**

TnI levels are elevated above normal in many patients presenting with unstable angina. There is a growing view that patients with unstable angina and elevated TnI represent a higher risk group and should be treated with platelet receptor antagonists.

**Prognostic Use**

TnI has significant prognostic usefulness in unstable angina patients. Patients presenting with unstable angina who had TnI levels above the normal range had 5 – 10 times greater risk of MI or death than patients with normal TnI levels (see references 4 and 5 in Summary section).

**Reference Ranges**

Due to the lack of standardisation of TnI assays, reference ranges vary considerably and it is essential to use the reference range and diagnostic cut-off points which have been established for the particular assay used.

International Federation of Clinical Chemistry (IFCC) and National Academy of Clinical Biochemistry (NACB) recommend two diagnostic cut-off's, a higher one for MI and lower one (usually equivalent to the upper limit of normal) for unstable angina (see references 1 and 2 in the Summary section).

European society for Cardiology (ESC) and the American College of Cardiology (ACC) recommend a single cut off at the 99th percentile of the reference population for diagnosis of MI (see reference 3 in the Summary section).

The consensus is that the single cut off approach be used only if the imprecision of the assay is <10%. Since the assays currently available cannot comply with this standard of precision, the two cut-off approach must be preferred at present.

**References**

1. Mair J, Morandell D, Genser N, Lechleitner P, Dienstl F, Puschendorf B. Equivalent early sensitivities of myoglobin, creatine kinase MB mass, creatine kinase isoform ratios, and cardiac troponin I and T for acute myocardial infarction. Clin Chem 1995;41:1266-72.
2. Heeschen C, Goldmann BU, Langenbrink L, Matschuck G, Hamm CW. Evaluation of a rapid whole blood ELISA for quantification of troponin I in patients with acute chest pain. Clin Chem 1999;45:1789-96.
3. Ebell MH, Flewelling D, Flynn CA. A systematic review of Troponin T and I for diagnosing acute myocardial infarction. J Fam Pract 2000; 49:550-556.

## **Cardiac Troponin T**

### **Physiology**

Troponin T (TnT) is a 39kD protein, which binds the Troponin complex (Troponins T, I, and C) to the tropomyosin strand in the contractile apparatus of muscle. There are a number of cardiac isomers of TnT which have 40 - 45% dissimilarity with skeletal muscle TnT. 6 - 8 % of cardiac TnT is found in the cytoplasm and the remainder is found complexed in tropomyosin.

### **Time course of release**

TnT becomes elevated 4 - 9 hours following an MI and remains elevated for about eight days. Levels may increase more than one hundred times the upper limit of values found in normal healthy subjects. Smaller increases may be found in patients presenting with unstable angina.

### **Analytical Factors**

Due to patent restrictions, assays for TnT are available from only one manufacturer (Roche; original assay from Boehringer).

While the first version of this immunoassay had 2% cross reactivity with skeletal TnT, improved versions of the kit are now available with improved analytical specificity.

TnT is relatively stable in the circulation.

TnT values measured in heparinised plasma are lower than those measured in serum; therefore, serum and plasma specimens are not interchangeable.

### **Diagnostic use - Clinical Sensitivity and Specificity**

#### **Myocardial infarction**

Clinical sensitivity of TnT for acute myocardial infarction is 90 - 100 % at 12 - 24 hours following the event (Katus et al). Estimates of the early sensitivity of TnT vary.

46% sensitivity 0-4 hrs after onset of chest pain, similar to TnI, myoglobin and CKMB mass (Mair et al)

Sensitivity of greater than 90% at 10 hours post chest pain and specificity of 87% and 80% at 1-10 hours post chest pain (Ebell et al).

#### **Renal Failure**

TnT has been reported to be elevated in patients with renal failure. Some authors report increased cardiac events in renal failure patients with increased TnT.

#### **Unstable angina.**

TnT concentrations are elevated in many patients presenting with unstable angina. There is a growing consensus that patients with unstable angina and elevated TnT represent a higher risk group than those with normal values and that the Troponin value should influence the treatment regimen.



**Prognostic Use**

TnT has significant prognostic usefulness in unstable angina patients. Patients presenting with unstable angina who had TnT concentrations above the normal range had 5 - 10 times greater risk of MI or death than patients with normal TnT levels.

TnT concentrations have been reported to correlate well with infarct size, but cardiac markers should not be routinely used for infarct sizing because they are inaccurate in the presence of spontaneous, pharmacological or surgical reperfusion.

**Reference Ranges**

Although TnT assays are standardised to a much greater extent than TnI assays, various discrimination limits for myocardial infarct have been used. It is therefore essential to use the reference range and diagnostic cut-off points recommended by the laboratory providing the assay.

The use of two diagnostic cut-offs is preferred at present: a higher value for diagnosis of MI and a lower value (usually equivalent to the upper limit of normal) to indicate greater risk in unstable angina patients. This approach is identical to that recommended for TnI.

**References**

1. Katus HA, Remppis A, Neumann FJ, Scheffold T, Diederich KW, Vinar G, Noe A, Matern G, Kuebler W. Diagnostic efficiency of troponin T measurements in acute myocardial infarction. *Circulation* 1991;83:902-12.
2. Mair J, Morandell D, Genser N, Lechleitner P, Dienstl F, Puschendorf B. Equivalent early sensitivities of myoglobin, creatine kinase MB mass, creatine kinase isoform ratios, and cardiac troponins I and T for acute myocardial infarction. *Clin Chem* 1995;41:1266-72.
3. Ebell MH, Flewelling D, Flynn CA. A systematic review of Troponin T and I for diagnosing acute myocardial infarction. *J Fam Pract* 2000; 49:550-556.

## **Myoglobin**

### **Physiology**

Myoglobin is a single polypeptide (153 amino acids) haem protein found in the cytosolic fraction of both cardiac and skeletal muscle tissue. Myoglobin is catabolised by glomerular filtration, proximal renal tubular absorption through endocytosis and proteolysis.

Myoglobin is currently the earliest biological marker of myocardial necrosis. Its low molecular weight (17.8 kDa) gives it a quicker diffusion in the circulating blood than enzymes such as CK or LDH.

### **Time Course**

Myoglobin appears in the peripheral blood 2-3 hrs after pain. Peak levels of myoglobin are reached after 6-9 hrs and return to normal within 18-36 hours.

### **Clinical Sensitivity and Specificity**

Sensitivity of myoglobin at presentation is 44-55%, increasing to 74-82% at 3-4 hours post presentation. If myoglobin is normal on admission, then 90 minutes later, taking a myoglobin cut-off concentration of 100 µg/L, a sensitivity of 100% and a specificity >90% has been reported.

### **Analytical factors**

High imprecision and marked disagreement among commercial myoglobin assays need to be considered when establishing reference ranges. Different manufacturers report different stabilities. Interference by heterophilic antibodies and Rheumatoid Factor appear to be method specific.

### **Diagnostic use**

There is now a consensus that myoglobin can be used as an early marker, but should be used in conjunction with a cardiac specific marker such as Troponin I or T. Due to rapid elimination by the kidney the steep increase in serum myoglobin concentration is followed by a rapid fall so that values within the “normal” range can be found about 24 hours (possibly 12 hours, which could reflect the assay used) after the infarct in an uncomplicated case, and can be missed when there is a delay in presentation. This normalisation will allow the very early detection of a recurrence or an MI extension. Reduced renal clearance results in elevation of serum myoglobin. Increased levels of myoglobin can also be found in the following: exertion, severe shock, extensive trauma, serious intoxication, terminal stage of kidney insufficiency, myocarditis, acute infectious diseases, and myopathies.

**Prognostic use**

It has been suggested that myoglobin measurement can be used to monitor thrombolytic success, and that there is a slower rise in level with poor perfusion.

The most reliable indicator of reperfusion is suggested to be an index of myoglobin levels between the level at baseline and 2 hrs post initiation of thrombolysis (diagnostic efficiency of 85%).

**Reference ranges**

Reference ranges vary according to age, sex, ethnic group and assay used but are approximately as follows.

Men < 80 µg/L (ng/ml)

Women < 60 µg/L (ng/ml)

**References**

1. Apple FS. Acute myocardial infarction and coronary reperfusion. Serum cardiac markers for the 1990's. Am J Clin Pathol 1992;97:217-226.
2. Apple FS, Christenson RH, Valdes Jr R, Andriak AJ, Duh SH, Feng YJ, Koplen B, Jortani SA, Johnson NA, Berg A, Mascotti K, Wu AHB. Simultaneous rapid measurement of whole blood myoglobin, creatine kinase MB, and cardiac Troponin I by the Triage Cardiac Panel for Detection of Myocardial Infarction. Clin Chem 1999;45:199-205.

## Summary

### **Diagnostic approach to the use of biochemical cardiac markers in acute coronary syndromes**

Cardiac Troponin (I or T) is the new standard for diagnosis of myocardial infarction and detection of myocardial cell injury. For patients presenting with cardiac chest pain who have elevated ST segment changes on ECG, biochemical markers are not necessary for diagnosis but can be used to confirm the diagnosis. For the remaining patients presenting with ischaemic symptoms, with or without acute ischaemic changes on ECG, the following approach represents consensus guidelines from the European Society for Cardiology/American College of Cardiology, International Federation of Clinical Chemistry and Laboratory Medicine, and US National Academy of Clinical Biochemistry. The recommended approach depends upon the hospital's arrangement for triage and admission.

### **Routine Triage**

For hospitals that do not have an area for rapid triage, a very early marker is unnecessary and a single definitive marker with high sensitivity and specificity such as Troponin I or T should be used. Troponin should be measured on presentation to A&E, 6 - 9 hours later and if necessary, 12 - 24 hours following presentation. When care of the patient does not require rapid cardiac marker testing, measuring Troponin at presentation and 12 - 24 hours later is more cost-effective.

### **Rapid Triage**

For hospitals with the protocol and facilities for rapid AMI rule-out, two biochemical markers should be used: an early marker, reliably increased in blood within six hours of symptoms, e.g. myoglobin, plus a definitive marker with high sensitivity and specificity, e.g. Troponin I or T. In this situation both markers should be measured on presentation to A&E, at 2 - 4 hours, and 6 - 9 hours following presentation. If necessary, Troponin should be measured again 12 - 24 hours following presentation.

Note:

1. Acute myocardial infarction or myocardial injury should not be ruled out on the basis of the results of a single specimen or test result.
2. It is not necessary to complete the sampling sequence above if the Troponin results and ECG are clearly positive for acute myocardial infarction.
3. If the result of an early specimen is negative or in the intermediate risk range, a later blood specimen for Troponin should be taken to identify patients with evolving MI.

Measurement of AST, LDH and its isoenzymes are no longer recommended. Total CK may have a role if combined with measurement of CK-MB. However the latter should be measured by immunoassay (CK-MB mass) rather than by immuno-inhibition assay. Cardiac Troponin assays are preferred to CK-MB mass assays because they are more specific, while being of similar cost.

The use of two diagnostic cut-off points has been recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the US National Academy of Clinical Biochemistry (NACB): a higher value for diagnosing MI and a lower value, usually equivalent to the upper limit of normal, to identify patients at greater risk of further cardiac events. The European Society for Cardiology/American College of Cardiologists recommend a single cut-off point, at the 99 percentile of the reference population, for diagnosis of MI. The latter approach is based upon the view that unstable angina and myocardial infarction represent a continuum rather than two separate entities. However, there is a widespread view that the latter approach should only be implemented if the imprecision of the assays at this cut-off point is less than 10%. Since the assays currently available do not comply with this standard of precision, the two cut-off approach is preferred at present.

### References

1. Panteghini M et al. Use of biochemical markers in acute coronary syndromes. Position paper of the IFCC Committee on Standardisation of Markers of Cardiac Damage. Clin Chem Lab Med 1999; 37: 687-693.
2. Wu AHB et al. National Academy of Clinical Biochemistry Standards of Laboratory Practice: Recommendations for the use of Cardiac Markers in Coronary Artery Diseases. Clinical Chemistry, 1999;45: 1104-21.
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## **BNP (Brain Natriuretic or B-type Natriuretic Peptide)**

### **What is BNP?**

The heart releases two natriuretic peptides, ANP (Atrial Natriuretic Peptide, secreted primarily by the atrial myocardium in response to dilatation), and BNP (Brain Natriuretic Peptide), manufactured and secreted almost exclusively by ventricular myocardium in response to elevations of end-diastolic pressure and volume). C-type Natriuretic Peptide is produced and released by endothelial cells in response to shear stress. BNP has potent natriuretic as well as diuretic and vasodilator effects.

BNP is synthesised as pro-BNP (1-108 amino acids), which is cleaved between amino acid 76 and 77 to yield a 77-108 aminoacid carboxyterminal fragment, the active hormone, referred to as BNP, and an amino terminal fragment of 1-76 aminoacids referred to as NT-proBNP (or N-BNP, or proBNP (1-76)). All three forms of BNP are released into the blood stream, where BNP is cleared by a clearance receptor (NPR-C), and an endopeptidase (NEP) resulting in a  $t_{1/2}$  of 20 minutes. In contrast to BNP, no specific elimination system for NT-proBNP has been found and consequently its slow clearance from the circulation results in an extended  $t_{1/2}$  of 60-120 minutes, and a much higher circulating level than BNP.

### **Why measure BNP or NT-pro-BNP?**

Congestive Heart Failure (CHF) is a common condition with high morbidity and mortality, and with an asymptomatic phase that when treated can reduce mortality and progression. CHF is a clinical syndrome of diverse aetiology (e.g. myocardial or ventricular dysfunction, valvular stenosis or incompetence) and to date there have been no markers available to determine whether a patient has CHF (classification is by the symptom based New York Heart Association classification), to measure its severity and to monitor therapy. A normal BNP virtually excludes Left Ventricular Systolic Dysfunction (LSVD), whereas an elevated BNP indicates the presence of cardiac disease and the need for an echocardiogram. BNP level correlates with the severity of heart failure, but whilst indicative of LSVD, it is also elevated in other cardiovascular conditions such as pulmonary hypertension and right ventricular overload. BNP predicts mortality in chronic heart failure and ventricular dysfunction and survival in acute coronary syndromes. The same claims are made for NT-proBNP.

### **BNP and NT-proBNP Assay:**

A point-of-care assay for BNP in blood has been FDA approved, but Coefficients of variation are typically >15%. As BNP is unstable, and normally in low concentration, sample requirements for other BNP assays, with and without extraction, are assay dependent. An automated NT-proBNP method is available for the Roche Elecsys analyser. This assay, which employs both aminoterminal and carboxyterminal directed antisera, shows complete stability of NT-proBNP in whole blood over three days at room temperature, irrespective of whether the blood is collected in a clotted, heparinised or EDTA tube, and claims Coefficients of variation <5%. The good stability of NT-proBNP is a significant advantage compared to that of BNP.

### **Reference ranges**

Diagnostic and prognostic levels of BNP and NT-proBNP are still under discussion. Antibodies raised against various epitopes are used by different manufacturers, and concentrations of both BNP and NT-proBNP may be method dependent until some standardization is established. The issue of fragment cross-reactivity in immunoassays could confound interpretation. Concentrations appear to increase with age, but this may not be significant.

### **References**

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**PART II**  
**RISK FACTORS**

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## **Total Cholesterol**

### **Physiology**

Cholesterol is essential for fluidity and viability of all biological membranes and is a precursor of bile acids, vitamin D, and other steroids. All cholesterol is transported in plasma in lipid-protein complexes. High concentrations of total cholesterol are strongly associated with premature atherosclerosis and coronary heart disease.

### **Analytical Factors**

#### **Variation.**

Intraindividual variation in cholesterol concentrations requires that it be measured on at least three occasions one week apart, within two months. The mean result should be used.

#### **Is Fasting required?**

Total cholesterol (as a single test) is a satisfactory measure in non-fasting persons.

When part of a lipid profile, fasting for at least 12 hours is necessary.

#### **Sampling Requirements**

Patient should have blood taken in a sitting position after a 5-minute rest.

Acceptable specimens are clotted or heparinised blood.

Elevated bilirubin, haemolysis, and lipaemia can interfere with many methods.

#### **In-Vivo Interferences**

Post MI: Cholesterol concentrations are lowered by the acute phase response, but particularly after a myocardial infarction (MI), major surgery or trauma.

Measurements may be taken on admission post-MI. Thereafter measuring cholesterol concentration is not recommended for a period of 3-4 months. Note that although a 'low' cholesterol level during this period may be inaccurate, a raised cholesterol generally indicates a need for lipid-lowering treatment.

Other: Pregnancy, drugs, obesity or smoking can raise blood cholesterol.

### **Reference Ranges**

Consensus of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III):

Desirable	< 5.2 mmol/L
Borderline high	5.2 to 6.2 mmol/L
High	> 6.2 mmol/L

**Risk Assessment**

Prospective studies have shown that incidence of CHD rises continuously with blood total cholesterol. This is steeper at higher cholesterol levels. Evidence from prospective trials shows that lowering concentrations of total cholesterol in patients with CHD slows or reverses the progression of the disease.

After the second decade cholesterol concentrations increase progressively. Whether this is physiological or a manifestation of increasing coronary disease with age is a matter of debate.

**Recommendations**

NCEP Guidelines are given in the reference range section above.

The Joint British Recommendations on Prevention of Coronary Heart Disease in Clinical Practice and the European Societies of Cardiology, Atherosclerosis and Hypertension Task Force Statement recommend that a plasma cholesterol consistently below 5.0 mmol/L should be the goal for patients with existing CHD or other atherosclerotic disease, and high risk patients without overt disease.

A Coronary Risk Chart has been produced by the latter group which defines risk by incorporating cholesterol, age, blood pressure and smoking habit.

**Reference**

1. National Cholesterol Education Programme. Third Report of the Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (NCEP ATP III) Executive Summary. JAMA 2001;285:2486-2497  
<http://www.nhlbi.nih.gov/guidelines/cholesterol/>.
2. The Joint British Recommendations on Prevention of Coronary Heart Disease in Clinical Practice and the European Societies of Cardiology, Atherosclerosis and Hypertension Task Force Statement. Heart 1998;80 (supplement 2):S1-S29.

## High Density Lipoprotein-Cholesterol (HDL-C)

### Physiology

HDL, the smallest lipoprotein, contains a lipid core of cholesterol and triglyceride surrounded by phospholipids and apoproteins (predominantly ApoA1).

It is a source of apoproteins for chylomicrons and very low density lipoprotein and it mediates reverse cholesterol transport, i.e. transportation of cholesterol from extrahepatic tissues to the liver for excretion.

### Analytical Factors

#### Variation.

Intraindividual variation indicates that at least two measurements should be taken at least one week apart. The mean result should be used.

#### Is Fasting required?

Fasting for at least 12 hours is necessary.

#### Sampling Requirements:

Serum is recommended, but some laboratories will accept heparinised specimens.

Patient should have blood taken in a sitting position after a 5-minute rest.

Bilirubin, haemolysis, and lipaemia can interfere with the commonly used assays.

Sample should be analysed within 2 days of collection.

#### In-Vivo Interferences

Post MI: HDL-C concentrations change minimally during the first 2 days but show a significant fall at 9 days. Concentrations do not fully return to pre-MI values for at least 3 months.

Similarly HDL-C should not be measured for at least 3 months post severe infection or inflammation.

Other factors that affect HDL-C concentration include obesity, lack of exercise, hypertriglyceridaemia, and drugs.

### Reference Ranges

	HDL	HDL: Total Cholesterol	
Higher risk	< 1.03 mmol/L	< 0.15	(increased risk factor of CHD)
Desirable	> 1.53 mmol/L	> 0.25	(decreased risk factor of CHD)

**Risk Assessment**

Plasma concentrations of HDL-C correlate inversely with the presence of CHD.

A low HDL-C concentration is an independent risk factor for CHD. At any given concentration of total cholesterol the relative risk of CHD increases with decreasing concentrations of HDL-C.

Although low HDL-C concentrations may occur as an isolated abnormality, they are frequently associated with raised concentrations of plasma triglycerides. This is the predominant lipid abnormality seen in type 2 diabetes.

The incidence of MI is increased almost 2-fold in men with HDL-C concentrations < 1.3 mmol/L and more than 4-fold in women with similar concentrations.

For every 0.025 mmol/L rise in HDL-C, the risk of CHD decreases by 2% in men and 3% in women. This relationship is independent of the total cholesterol concentration.

**Reference**

1. National Cholesterol Education Programme. Third Report of the Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (NCEP ATP III) Executive Summary. JAMA 2001;285:2486-2497.  
<http://www.nhlbi.nih.gov/guidelines/cholesterol/>.

## **Low Density Lipoprotein-Cholesterol (LDL-C)**

### **Physiology**

LDL is a small, cholesterol-rich lipoprotein, which contains apoB as the major apoprotein. It accounts for about 70% of total cholesterol in plasma. LDL particles are taken up either by the liver or by peripheral cells through a receptor mediated process. Free cholesterol is then liberated from LDL and accumulates within cells.

Oxidised LDL particles in vessel walls can attract macrophages, which become foam cells as they ingest LDL particles. This is believed to be one of the initiating steps in atherosclerosis.

### **Analytical Factors**

#### **Variation.**

Intraindividual variation indicates that at least two measurements should be taken at least one week apart. The mean result should be used.

#### **Is Fasting required?**

Fasting for at least 12 hours is necessary.

#### **Sampling Requirements:**

Serum or plasma sample is recommended.

Patient should have blood taken in a sitting position after a 5-minute rest.

Specimens can be stored at -4°C for up to 3 days and for several weeks at -20°C.

#### **In-Vivo Interferences:**

Post MI, stroke, pregnancy or lactation: samples should not be taken for at least 3 months.

Other factors that influence LDL-C concentration include dietary changes, obesity, smoking, alcohol, exercise, acute infections, drugs (e.g., antihypertensives).

#### **Secondary causes of hyperlipidaemia .**

Hypothyroidism and diabetes are the most common secondary causes of raised LDL-C.

#### **Calculation / Direct Measurement.**

Traditional LDL-C estimation (in mmol/L) uses the Friedewald equation ( $\text{LDL-C} = \text{total cholesterol} - \text{HDL-cholesterol} - \text{triglyceride}/2.2$ ). The accuracy of the estimation is limited by analytical errors in the individual measurements. It is not accurate when chylomicrons are present, if non-fasting samples are used, or in Type III Dyslipoproteinaemia.

Methods for direct measurement of LDL-C have become available.

**Reference Ranges (NCEP ATP III guidelines)**

Optimal: < 2.6 mmol/L,  
Near optimal: 2.6-3.3 mmol/L,  
Borderline high: 3.4-4.1 mmol/L,  
High: 4.2-4.9 mmol/L,  
Very high: > 5.0 mmol/L.

**Risk Assessment**

LDL is the major atherogenic lipoprotein and has long been identified by as the primary target of cholesterol-lowering therapy. This focus on LDL has been strongly validated by recent clinical trials, which show the efficacy of LDL-C lowering therapy for reducing risk for CHD.

The positive relationship between serum cholesterol levels and the development of CHD is observed over a broad range of LDL-C levels; the higher the level, the greater the risk.

Recent clinical trials indicate that a 1 percent decrease in LDL-C reduces risk of developing CHD by about 1 percent. Risk reduction is greatest when early intervention occurs.

The Joint British Recommendations on Prevention of Coronary Heart Disease in Clinical Practice and European Societies of Cardiology, Atherosclerosis and Hypertension Task Force Statement recommend that patients with existing CHD or other atherosclerotic disease, and high risk patients without overt disease plasma LDL-C should be consistently below 3.0 mmol/L.

**References**

1. National Cholesterol Education Programme. Third Report of the Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (NCEP ATP III) Executive Summary. JAMA 2001;285:2486-2497.<http://www.nhlbi.nih.gov/guidelines/cholesterol/>.
2. The Joint British Recommendations on Prevention of Coronary Heart Disease in Clinical Practice and the European Societies of Cardiology, Atherosclerosis and Hypertension Task Force Statement. Heart 1998;80 (supplement 2):S1-S29.

## Triglycerides

### Physiology

Triglycerides are water insoluble lipids consisting of 3 fatty acids linked to one glycerol molecule. They are transported in blood as core constituents of all lipoproteins. They are present in greatest concentrations in chylomicrons (synthesised in the small intestine) and very low density lipoproteins (VLDL) (synthesised in the liver). Concentrations can be elevated in diabetes, hypothyroidism, renal disease, alcoholism, and viral infections.

### Analytical Factors

A 12-hour fasting sample is required.

Patients should be seated for at least 5 minutes before sampling.

Intraindividual variation requires that at least two measurements should be taken at least one week apart. The mean result should be used.

Samples are stable for about 3 days at 2-4°C, several weeks at -20°C, and several years at -50 to -80°C.

Post MI or post surgery triglyceride concentrations can be markedly increased. Concentrations do not fully return to pre-MI values for at least 3 months.

Concentrations are affected by drugs (including thiazide diuretics), diet, obesity, exercise, alcohol and smoking.

### Reference Ranges (NCEP ATP III guidelines)

Optimal	<1.7 mmol/L
Borderline high	1.7-2.2 mmol/L
High	2.3-5.6 mmol/L
Very high	>5.6 mmol/L

### Risk Assessment

The European Societies of Cardiology, Atherosclerosis and Hypertension Task Force Statement state that triglyceride concentrations >2.0 mmol/L identify those at higher risk for CHD.

The Joint British Recommendations on Prevention of Coronary Heart Disease in Clinical Practice state that plasma triglyceride concentrations above 2.3 mmol/L in a fasting sample signals the need for an investigation of secondary causes.

The importance of an isolated triglyceride elevation remains controversial and there is little consensus for screening.

HDL-C concentrations are usually low when triglycerides are elevated.

Drug therapy of hypertriglyceridaemia may be beneficial in reducing the risk of cardiac events although secondary causes of hypertriglyceridaemia would usually be treated before initiating therapy.

Weight reduction and regular exercise lower triglyceride concentrations.

Patients with extremely high triglyceride concentrations (>11.0 mmol/L) are at risk for developing acute pancreatitis.



**References**

1. Forrester JS. Triglycerides: Risk factor or fellow traveller? *Curr Opin Cardiol* 2001;16:261-4.
2. National Cholesterol Education Programme. Third Report of the Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (NCEP ATP III) Executive Summary. *JAMA* 2001;285:2486-2497.  
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3. The Joint British Recommendations on Prevention of Coronary Heart Disease in Clinical Practice and the European Societies of Cardiology, Atherosclerosis and Hypertension Task Force Statement. *Heart* 1998;80 (supplement 2):S1-S29.

## **Lipoprotein(a) (Lp(a))**

### **Physiology**

Lp(a) is a low density lipoprotein-like particle formed by the association of the highly polymorphic glycosylated apolipoprotein(a) with apolipoprotein B-100, the predominant protein moiety of LDL.

Apo(a) is synthesised in the liver. Its concentration is strongly influenced by genetic factors. There are many distinct isoforms.

### **Analytical Factors**

Lp(a) should be measured in fresh serum specimens because samples degrade over time even when frozen.

A fasting sample is not required if Lp(a) is measured in isolation.

Lp(a) is much less affected by age, gender, weight and diet than other classes of lipoprotein.

Intraindividual variation is negligible and Lp(a) concentrations are relatively stable throughout life.

Interindividual concentrations can vary over 1000-fold.

Lp(a) concentrations are affected by severe liver disease, hypertriglyceridaemia, renal transplantation, inflammation and thyroid disorders.

### **Reference Range**

The recommended cut-off for Lp(a) is 1.07  $\mu\text{mol/L}$ , representing the 80th percentile of the general population.

### **Risk Assessment**

Retrospective studies have shown a strong association between Lp(a) and CHD. Prospective studies demonstrate a more modest association.

The relationship between Lp(a) concentration and CHD risk appears stronger in men than women. The risk appears to be greater among younger individuals (< 60 years). Elevated concentrations may not be a risk factor in the elderly.

Methodological problems, lack of evidence demonstrating isolated reduction of Lp(a) is beneficial, and limited pharmacological therapies, suggest screening of the general population is not recommended. It has been recommended that the detection of Lp(a) concentrations should be reserved for individuals with premature MI in the context of a relatively normal risk-factor profile, those with familial hypercholesterolaemia, or those with a very high coronary risk.

In high-risk individuals the atherogenic potential of Lp(a) can be reduced by aggressive reduction of LDL-C.

### **Reference**

Wieringa G. Lipoprotein(a): what's in a measure? *Ann Clin Biochem.* 2000;37:571-80.

## **Second Line Tests**

### **Lipoprotein Electrophoresis**

There is rarely any requirement for Lipoprotein Electrophoresis, the primary use being in confirming the presence of IDL (Broad Beta) in Fredrickson's Type III Dyslipoproteinaemia.

### **Apolipoproteins A1 and B**

Since the advent of agreed International Standardisation of the measurement of Apolipoproteins (Apo) A1 and B-100), these assays have gained in usefulness.

Apo B meets the criteria establishing it as a "causative" agent in the atherosclerotic process. It has been shown that pharmacological intervention to decrease levels reduces the number of CHD-related events and produces the regressions of atherosclerosis.

Apo A1 is the major protein constituent in HDL. One major role is the activation of Lecithin Acyl Transferase (LCAT). It also has a role in Cholesterol efflux from cells and with the HDL-Receptor in the liver.

### **Apolipoprotein E Phenotyping (or Genotyping )**

VLDL and Chylomicron remnants are removed from the circulation by the liver using a specific Apo E Receptor. There are three main inherited forms - E2 (8% of Caucasians), E3 (78%) and E4 (14%). If E3 and E4 are considered to have 100% specificity for the receptor, E2 has only 2%. Patients who are homozygous for E2 may develop Fredrickson's Type III Dyslipoproteinaemia. They have an increased risk of CHD and Peripheral Vascular Disease, especially stroke.

Subjects with E4 in their genotype are particularly sensitive to the inclusion of Cholesterol (meat and dairy products) in their diet but not to total fat content. The remainder of the population must reduce the total fat content of the diet to lower lipids. These findings, from a Scandinavian Study in the early eighties, have profound consequences for the dietary management of patients with lipid disorders. There are possible medico-legal complications to the determination of E Phenotype or Genotype as there is a reported tendency to develop Alzheimer's Disease in those subjects who have E4 in their genetic make-up.

## C-reactive Protein (CRP)

### Physiology

C-reactive protein (CRP) consists of five identical polypeptide subunits with a total molecular weight of about 120 kDa. CRP is an acute phase reactant. Its concentration in serum is markedly increased (in the range 5-1,000 mg/L) during acute and chronic inflammation, acute infection, trauma or neoplastic proliferation. Otherwise, plasma CRP concentrations are physiologically stable over long time periods. There is no significant circadian rhythm.

A systemic low-grade inflammatory response is an integral part of the atherosclerotic process, and is associated with a small increase in baseline serum CRP concentration (in the range 0.1-10 mg/L). High-sensitivity CRP (hs-CRP) assays have been developed which can be used to detect these small increases, and there is strong evidence that these measurements could be useful as a prognostic indicator in patients with established coronary disease, and as a predictor of future coronary disease in apparently healthy men and women.

### Analytical Factors

hs-CRP assays have detection limits of about 0.15 mg/L in plasma, corresponding to the 2.5<sup>th</sup> percentile of normal population values. For cardiac disease risk stratification, assay imprecision of <10% at a CRP concentration of 0.2mg/L is required. At present, measurements are not consistent between various methods. No primary reference method exists and no primary reference material has been developed.

Serum or plasma (EDTA or lithium heparin anti-coagulant) samples are suitable. CRP is stable in whole blood samples for 48h at 4°C and at 20°C. hs-CRP should be measured when the individual is apparently healthy, with no active or recent infection or inflammation. Two measurements should be made 3-4 weeks apart, and the lower one used for risk assessment. Values of hs-CRP >10mg/L suggest active inflammation, and should be repeated after 3-4 weeks.

### Reference Ranges

	<u>Median (mg/L)</u>	<u>0.95 interval (mg/L)</u>
General population	0.58-1.14	0.08-3.11

Plasma hs-CRP concentrations in the general population show a non-Gaussian distribution, and are heavily skewed to lower values. The median hs-CRP increases with age, smoking, in subjects with osteoarthritis, and in the obese.

### Risk Assessment

Several studies have shown that plasma hs-CRP measured at admission or discharge of patients with acute coronary syndromes may have prognostic significance. Values of hs-CRP concentration >3mg/L were associated with increased incidence of recurrent angina, coronary revascularisation, MI or cardiovascular death, within 6 months, regardless of cardiac Troponin I concentration, and hs-CRP >15.5 mg/L was a strong predictor of 14-day mortality.

In ten prospective studies of an overall total of more than 5,000 apparently healthy men and women in Europe and the U.S.A., plasma hs-CRP concentration was a strong predictor of future MI or stroke. Tables of relative risk estimates for future cardiovascular disease have been published using hs-CRP cut-points based on quintiles of the population range (i.e. < 0.7, 0.7-1.1, 1.2-1.9, 2.0-3.8, 3.9-15.0 mg/L), with risk increasing more than two-fold from the lowest to the highest quintile. Whereas hs-CRP is an independent risk factor in these studies, it is of greater use when assessed in conjunction with the total cholesterol : HDL-cholesterol ratio. Although these findings have been consistent in all epidemiological studies, the accuracy, for classification of future risk, of a single hs-CRP measurement in an individual patient is uncertain, because of the within-subject CV of 40%. It has been suggested that a simpler classification into high risk and low risk groups, with an intermediate grey area, may prove to be more practical. This is a rapidly evolving area of investigation, and cut-points are likely to be modified.

### References

1. Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. Clin Chem 2001; 47:403-411.

2. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, Gallimore JR, Pepys MB. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ* 2000; 321:199-204.
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## Homocysteine

### Biochemistry

Homocysteine is a sulfhydryl amino acid with essential roles in methionine metabolism and cysteine biosynthesis. Vitamins B<sub>12</sub>, B<sub>6</sub>, and folate, are essential cofactors for several of the enzymes. During synthesis from methionine in the 'activated carbon cycle' 5-methyl-tetrahydrofolate is first generated, then reconverted to tetrahydrofolate (THF) in a B<sub>12</sub> dependent reaction catalysed by methionine synthase (MS). Regeneration of 5-methyl-THF depends on methylene tetrahydrofolate reductase (MTHFR) activity. Synthesis of cysteine is achieved in two steps catalysed by cystathionine β-synthase (CBS) and cystathioninase, which are B<sub>6</sub> dependent.

Hyperhomocysteinaemia occurs in: hereditary deficiencies of CBS, MS, MTHFR; hereditary disorders of cobalamin metabolism; deficiencies of folate, and vitamins B<sub>12</sub> and B<sub>6</sub>.

Severe hyperhomocysteinaemia (>100 µmol/L) occurs in CBS deficiency (classic homocystinuria), an autosomal recessive trait (Irish incidence 1:65,000). Vascular thromboembolism (VTE), the major cause of morbidity and mortality, has been almost completely prevented by therapies which reduce plasma homocysteine concentration to normal or around 100 µmol/L.

MTHFR deficiency, including that due to a thermolabile variant, is associated with an increased incidence of VTE which may occur at any stage between neonatal and adult life.

A pathophysiologic mechanism for the clinical manifestations has not yet been identified. Experimental studies suggest possible effects of homocysteine on endothelial cells and on platelet adhesiveness.

### Analytical Factors

Total plasma homocysteine (tHcy) includes: protein bound (80-90%), homocystine dimer (5-10%), cysteine-homocysteine mixed disulfide (5-10%), and free (1%).

Homocysteine is synthesised by red blood cells at room temperature. To avoid false elevation of tHcy, collect blood from fasting patients into EDTA-tubes stored on ice. Separate plasma within one hour. Homocysteine is stable in plasma (days at room temperature, weeks at 0-2°C, years at -20°C).

**Reference Interval:** 5 - 15 µmol/L

### Clinical applications of tHcy:

- detection and monitoring of hereditary hyperhomocysteinaemia.
- detection of folate, vitamin B<sub>12</sub>, or B<sub>6</sub>, deficiency.

Hyperhomocysteinaemia is associated with: smoking; excess alcohol or coffee; lack of exercise; advanced age; renal failure; hypothyroidism; systemic lupus erythematosus; transplantation; medications including methotrexate, colestipol, nicotinic acid, theophylline, L-dopa, and exposure to nitrous oxide.

**Risk Assessment**

Obligatory heterozygotes for CBS deficiency, who may have normal or elevated tHcy, may be at increased risk of vascular abnormalities by age 41 to 50.

Risk of VTE may be increased in hyperhomocysteinaemic individuals who have coexistent coagulation defects, notably the Factor V Leiden mutation.

Some epidemiological studies demonstrated a statistically significant association of *mild* hyperhomocysteinaemia (16-30  $\mu\text{mol/L}$ ) with subsequent risk of myocardial infarction or vascular disease. The risk was considered independent of other cardiovascular risk factors. There is as yet no evidence of clinical benefits to the general population from reducing tHcy by dietary supplementation with folate, vitamins B<sub>12</sub> or B<sub>6</sub>. Trials are in progress to determine the effect of vitamin supplementation on the incidence of cardiovascular events in individuals with mild hyperhomocysteinaemia. Reports are expected in the early 2000's.

There is insufficient evidence at this time to support screening of the general population for mild hyperhomocysteinaemia as an independent risk factor for cardiovascular diseases.

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