

Recommended standards for biochemical markers of myocardial damage: All Wales Clinical Biochemistry Audit Group

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Abstract

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The effective use of cardiac-specific troponin estimations in the diagnosis of acute myocardial infarction (AMI) is clouded by the imprecise definition surrounding the decision limits. This has led to a wide variation of criteria for the diagnosis of myocardial infarction. A survey of troponin measurements in Welsh laboratories, undertaken in 2003 under the auspices of the All Wales Clinical Biochemistry Audit Group, revealed significant variations in laboratory and clinical practice. Extensive discussion and consultation led by a working group of clinical biochemists and cardiologists in Wales culminated in recommendations concerning the use of troponin assays to establish myocardial damage.

The key recommendations are:

- Cardiac troponin (T or I) should be the first-line test for myocardial damage;
- Two samples should be collected, at admission and 12–24 h later. The first sample is used for 'rule in' purposes, but not to 'rule out' myocardial damage;
- Only one threshold (cut-off) value for troponin should be quoted on laboratory reports, values above which are indicative of myocardial damage.

A study by the Wales External Quality Assurance Scheme (WEQAS) enabled the derivation of the recommended cut-off concentrations of troponin for defining myocardial damage, defined for each assay as the concentration that can be reliably distinguished, with a confidence interval of 99%, from the 99th percentile reference limit.

These recommended standards provide a rationale for a uniform approach for troponin assays for patients with chest pain, working towards a standardized approach to the diagnosis and management of patients presenting with acute coronary syndromes.

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Overview of current guidance

The 1979 WHO criteria for the diagnosis of acute myocardial infarction (AMI)¹ required two out of three of:

- Classical symptoms (particularly chest pain);
- Electrocardiographic (ECG) changes;
- Serial changes in enzyme markers, specifically creatine kinase (CK; total or MB isoenzyme).

The diagnosis of clinical AMI required sufficient myocardial necrosis to elevate CK and/or produce characteristic ECG changes. Other diagnostic labels such as non-ST elevation AMI (NSTEMI) and unstable

angina implied lesser changes in ECG and/or CK, all these diagnoses being associated with an adverse coronary prognosis. The same criteria were used initially to establish clinical decision points for cardiac troponin as for CK.^{2,3} Troponin cut-off points for myocardial infarction (MI) were high, resulting in the classification of patients who had suffered myocardial damage but who did not fulfil the WHO definition of AMI as having troponin-positive unstable angina or minimal myocardial damage. A 2004 discussion paper by a British Cardiac Society working group⁴ proposed that this old definition of AMI be re-invented.

It has now been shown that any detectable troponin elevation is associated with an increased risk of major

adverse cardiac events (MACE) and that this risk increases with the magnitude of the troponin elevation.^{5,6} A clinical continuum called Acute Coronary Syndrome (ACS) has now been recognized, together with the importance of detecting even a small amount of myocardial injury. It has also been recognized that an increased troponin concentration indicates the presence of myocardial damage, but does not in itself diagnose the cause of the damage.

In 2000, the European Society of Cardiology (ESC) and American College of Cardiology (ACC) issued a consensus document⁷ redefining AMI, placing greater emphasis on cardiac troponin to detect myocardial damage, thus effectively lowering the diagnostic threshold. This document stated that, for the diagnosis of AMI, there must be a rise and fall in the serum cardiac troponin concentration and at least one of the following:

- Ischaemic symptoms;
- New ECG changes (ST-T segment changes and/or Q-waves);
- A specific setting of coronary artery intervention (e.g. coronary angioplasty).

'Non-infarct' clinical settings associated with an elevated cardiac troponin concentration include heart failure, pulmonary embolism, tachyarrhythmias, trauma, surgery, myocarditis and renal impairment.

Cut-off levels

The ESC/ACC Consensus paper⁷ proposed that a cardiac troponin concentration exceeding the 99th percentile for a healthy population is positive for myocardial damage. However, this value is so low that current assays are not sufficiently reproducible or accurate at this low level. Therefore, it was recommended that the value closest to the 99th percentile that could be measured with 10% imprecision, as measured by coefficient of variation (CV), should be used.

Attempts to define the 99th percentile for cardiac troponin include:

- A study from Roche using their third generation troponin T assay (detection limit 0.010 µg/L); 19 of 1951 healthy persons (1%) had a concentration > 0.010 µg/L;
- A study by Venge *et al.*⁸ of troponin I in 758 healthy persons using the Beckman Access II assay (detection limit 0.010 µg/L); the 99th percentile in healthy subjects was 0.020 µg/L.

Several troponin assay comparisons have been reported,⁹ with various approaches to defining reference or cut-off limits:

- (1) *Report troponin concentration down to the assay detection limit:* accurate discrimination between 'minor' myocardial injury and analytical noise requires assays that have high precision at this low troponin concentration. Further assay improvement will be required before this goal is achieved. Due to lack of standardization, a method that has a low limit of detection does not imply a sensitive assay.
- (2) *Report down to a concentration corresponding to the 99th percentile concentration of a healthy reference population:* problems remain with assay imprecision at these low concentrations.
- (3) *Report down to a concentration corresponding to a CV of 20%, 'the functional sensitivity' of the assay:* although myocardial damage is diagnosed more frequently, there is decreased confidence in that decision limit because it is close to the detection threshold.
- (4) *Report down to a concentration corresponding to a CV of 10%:* this was initially proposed by Apple and Wu¹⁰ and is now recommended by the National Academy of Clinical Biochemistry¹¹ and the International Federation of Clinical Chemistry (IFCC).¹² The European Society for Cardiology⁷ and American College of Cardiology⁷ consensus document supports the view that the acceptable imprecision for troponin methods, as measured by CV at the 99th percentile decision limit, should be ≤ 10%.

Opinion differs about whether to use the 99th percentile reference limit or the lowest concentration associated with the 10% CV, with responses to the views of Apple *et al.*¹³ stimulating further discussions around the justification of choosing 10% or 20% CV over the 99th percentile. Panteghini *et al.*,¹² on behalf of the IFCC, evaluated the imprecision for cardiac troponin assays at low concentrations on various analysers and concluded that current assays were not able to achieve the 10% CV recommendation at the 99th percentile reference limit defined by the manufacturers.

If the lowest concentration at which the 10% CV is achieved is used as the cut-off level, however, this value will be relatively higher for a troponin assay with poorer precision at low concentrations. Therefore, the assay method used by a laboratory to measure troponin could determine whether or not a patient is classified as having biochemical evidence of myocardial damage, resulting in wide variation in diagnosis. At an All Wales Clinical Biochemistry Audit Group meeting in 2004, it was agreed as a compromise that in order to reduce the inter-assay variability in interpretation, the cut-off level chosen should be the concentration that can be reliably distinguished, with a confidence interval of 99%, from the 99th percentile reference limit.

Wales External Quality Assurance Scheme (WEQAS) study

WEQAS undertook a study in 2004 to establish the intra-laboratory variation (both within and between batch) at a range of troponin concentrations for all laboratories in Wales, in order to obtain data to establish cut-off concentrations. Five pools of human serum spiked with complexed troponin were prepared and stored at -70°C until dispatch. Five sets of each pool were dispatched to all Welsh laboratories and stored at -20°C until assay. A sample from each pool was assayed in duplicate on each of two runs on each day for five days, so each pool was assayed in duplicate on 10 occasions. Laboratories were asked to analyse samples as if they were patient's samples; calibration frequency and reagent lot numbers were laboratory-dependent. Analysis of the data from the study is summarized in Table 1 and discussed elsewhere in this issue.¹⁴

Recommended standards for biochemical markers of myocardial damage

The following standards are recommended, taking into account:

- Surveys of troponin measurements in Welsh laboratories presented at the All Wales Clinical Biochemistry Audit Group meetings in 1999 and 2003;
- A literature search to define the arguments around lower reference limits and cut-off values for troponin assays;
- Data obtained from the WEQAS study of the performance of troponin assays provided by each laboratory in Wales;
- Consensus points agreed by clinical biochemists and cardiologists.

Chest pain protocols

An appropriate local protocol should be developed jointly by admitting physicians, cardiologists, emergency department staff, clinical laboratory staff and hospital administrators, for the use of biochemical markers in the evaluation of patients with possible

ACS. It should facilitate both the diagnosis and exclusion of ACS, and should be applicable to patients on admission or to those who are already in hospital.¹¹

Choice of biochemical test: cardiac troponin (T or I) should be the first-line test

- Cardiac troponins are now the gold standard test for detecting myocardial damage.^{11,15,16} They have high sensitivity and specificity for myocardial injury and remain abnormal for several days after the onset of symptoms. Whether troponin T or I is measured will depend on analyser availability; troponin T has advantages over troponin I because of lack of troponin I assay standardization and lack of good analytical correlation between commercial troponin I assays.
- For detecting suspected re-infarction (up to 10 days after the first episode), collection of serial blood samples over three consecutive days for CK (or CK-MB) should be considered,¹¹ because of the more rapid fall of CK (cardiac troponins have much longer half-lives). For CK-MB, immunometric assays, which measure CK-MB mass, are preferable to immunoinhibition assays of CK-MB activity.¹⁷
- Traditional 'cardiac enzymes' (AST, HBD and LDH) no longer have any role in investigating myocardial damage,^{11,15} due to poor specificity and sensitivity and the need for serial samples.
- Serum myoglobin is an early marker of myocardial damage that is increased in blood within 6 h of onset of symptoms. However, it has a low specificity for cardiac damage and is only useful for ruling out AMI. It is of limited value in hospitals where triage decisions are not made within the first few hours after admission;¹¹ only cardiac troponin is then needed.

Relative roles of ECG and biochemical markers

In patients with characteristic ECG changes on admission (e.g. ST elevation), the diagnosis of AMI can be made and acute treatment initiated without immediate measurements of biochemical markers of cardiac damage.^{11,18}

Table 1 Troponin assay precision and recommended cut-off concentrations for defining myocardial damage – data from the WEQAS study*

Troponin assay	Laboratories (n)	Troponin at CV 10% ($\mu\text{g/L}$)	Troponin at CV 20% ($\mu\text{g/L}$)	Troponin distinguishable from normal with 99% confidence ($\mu\text{g/L}$)
Roche	9	0.02	<0.01	0.02
Beckman AccuTnI	5	0.11	0.02	0.07
Bayer Centaur	1	0.36	0.14	0.19
Bayer ACS	1	0.49	0.23	0.34

* The data relate to total imprecision (i.e. combined within- and between-batch imprecision). CV=coefficient of variation.

Timing of samples for troponin

- (a) Two samples should be collected, at admission and 12–24 h later. For each sample, the date/time of sample collection AND the date/time of the suspected cardiac event should BOTH be stated on the request form to aid correct interpretation.
- (b) For patients admitted with symptoms suggestive of ACS, time of admission is preferable to time of onset of symptoms as the reference point for determining when blood should be collected.^{11,16,19} However, if the time of onset of a single chest pain event is deemed reliable, this time may be considered more appropriate. Time of onset of symptoms is the only suitable reference point for patients who develop symptoms while in hospital.
- (c) The first sample is used for 'rule in' purposes, but not to rule out myocardial damage due to ACS. The second sample can be used as both a rule-in and a rule-out test. It can also be used for prognostic purposes as it gives some indication of the extent of myocardial damage. However, up to 30% of patients with typical unstable angina (some of whom have other features such as dynamic ECG changes) and a negative troponin result at 12–24 h may have critical coronary artery disease, requiring urgent revascularization. Therefore, a negative troponin at 12–24 h should not be used alone to categorize patients as low risk.²⁰
- (d) This two-sample procedure will also help to indicate those individuals suffering other chronic conditions affecting myocardial function (e.g. myocarditis, heart failure and renal impairment).

Specimen

Serum is preferred for both troponin T and I assays. For troponin T, heparin samples are unsuitable (they may give false-negative results). The manufacturer's kit leaflet should be consulted for further information about suitable sample types for their particular assay.

Assay availability

This should be agreed with the clinical users of the service. Troponin assays should be available daily, including weekends and public holidays. If only a daytime service can be provided, results should be available as early as possible in the day to facilitate prompt clinical decision-making and optimal bed utilization. Troponin results should be available within 2 h of venepuncture when this service is required by senior clinicians.

Analytical interferences

Significantly haemolysed samples are unsuitable for troponin T. Fibrin and cell debris can give

false-positive results with any sample type, particularly with some troponin I assays, as can heterophilic antibodies. Staff should be trained to recognize potentially artefactual results and take steps to investigate unexpected results inconsistent with clinical findings (e.g. simple dilution studies, gamma globulin addition to neutralize heterophilic antibodies²¹ and assay of a fresh sample). If such procedures fail to explain an anomalous result, samples should be sent to another laboratory that measures the analyte using different technology. Anomalous results should be reported to the equipment manufacturer.

Point-of-care testing (POCT) for troponin

This is not currently recommended, as the modified POCT assays available have inadequate sensitivity and precision.

Internal Quality Control (IQC) and External Quality Assurance (EQA)

- (a) Appropriate IQC procedures should be in place. Efforts should be made to obtain and estimate IQC material at concentrations close to diagnostic cut-off levels for each assay. For cardiac troponin, this should include IQC material with a value close to the lower 10% CV value of the assay.
- (b) Laboratories should participate in an accredited EQA scheme for each marker that they offer, where such a scheme is available.

Reporting of troponin results

- (a) The units for reporting results should be $\mu\text{g/L}$.
- (b) Only one threshold (cut-off) value for troponin should be quoted on laboratory reports, values above which are indicative of myocardial damage. While the actual troponin level is related to the extent of myocardial damage and risk,²² the relationship is a continuum. Differences in within- and between-assay standardization and calibration hinder reliable definition of equivalent higher second cut-off levels that correspond to the classical (pre-2000 WHO) definition of AMI.
- (c) The cut-off level should be defined as the concentration that can be reliably distinguished, with a confidence interval of 99%, from the 99th percentile reference limit. It is recognized that the values obtained in the WEQAS study (Table 1) will change as assays are improved.
- (d) Every laboratory in Wales using the same assay system should use the same cut-off level.
- (e) If fewer decimal points are used for reporting results in the laboratory computer system than are provided by the analyser, the laboratory

should ensure that results close to the cut-off are rounded up or down appropriately and that 'rounding' does not lead to misclassification.

- (f) The use of cumulative reports, to include all troponin results available on the same patient within the preceding week, is recommended where possible.

Interpretation

Laboratories should provide comments as appropriate on reports to remind non-specialist clinicians of the limitations of troponin assays. In particular:

- (a) The troponin level may not be increased within the first 12 h after myocardial damage.
- (b) An increased troponin level indicates myocardial damage, but not its cause. The diagnosis of ACS/AMI must be supported with appropriate symptoms and/or ECG changes. In the absence of supportive clinical findings, other causes of myocardial damage should be considered.
- (c) The absolute troponin level may not reliably indicate the extent of myocardial damage. Other non-biochemical parameters, such as left ventricular function (ejection fraction), are better for this purpose.

Conclusions

These standards provide a rationale for a uniform approach to troponin assays in the investigation of patients with chest pain. The clinical implications of troponin concentrations above these cut-off points are the subject of much current debate and require clarification to enable standardization of the diagnosis and management of ACS and other conditions. Adherence to the recommended standards outlined here will place all laboratories in Wales on the same basis to aid that standardization of approach. The remaining biochemical challenge will be to determine equivalent values of troponin T and troponin I for identifying the same degree of myocardial damage in all laboratories. The outcome of the IFCC working group on the standardization of troponin I is eagerly awaited and will aid harmonization of troponin results.

References

- 1 World Health Organisation. Report of the Joint International Society and Federation of Cardiology/World Health Organisation Task Force on Standardisation of Clinical Nomenclature and criteria for diagnosis of ischaemic heart disease. *Circulation* 1979; **59**: 607-9
- 2 Collinson PO, Moseley D, Stubbs PJ, Carter GD. Troponin T for the differential diagnosis of ischaemic myocardial damage. *Ann Clin Biochem* 1993; **30**: 11-6
- 3 Apple FS, Maturen AJ, Mullins RE, *et al.* Multicenter clinical and analytical evaluation of the AxSym troponin-I immunoassay to

- assist in the diagnosis of myocardial infarction. *Clin Chem* 1999; **45**: 206-12
- 4 Fox KAA, Birkhead J, Wilcox R, *et al.* British Cardiac Society Working Group on the definition of myocardial infarction. *Heart* 2004; **90**: 603-9 (*Ann Clin Biochem* 2004; **41**: 263-71)
- 5 James S, Armstrong P, Califf R, *et al.* Troponin T levels and risk of 30-day outcomes in patients with the acute coronary syndrome: prospective verification in the GUST-JV trial. *Am J Med* 2003; **115**: 178-84
- 6 Bertrand ME, Simoons ML, Fox KA, *et al.* Management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J* 2002; **23**: 1809-40
- 7 Joint European Society of Cardiology/American College of Cardiology Committee. Myocardial Infarction redefined – a consensus document for the redefinition of myocardial infarction. *Eur Heart J* 2000; **21**: 1502-13
- 8 Venge P, Lagerqvist B, Diderholm E, *et al.* Clinical performance of three cardiac troponin assays in patients with unstable coronary artery disease (a FRISC II substudy). *Am J Cardiol* 2002; **89**: 1035-41
- 9 Hickman PE, Koerbin G, Tate J. The reporting of cardiac troponin. *Clin Biochem Rev* 2003; **24**: 99-108
- 10 Apple FS, Wu AHB. Myocardial infarction redefined: role of cardiac troponin testing (editorial). *Clin Chem* 2001; **47**: 377-9
- 11 Wu AH, Apple FS, Gibler WB, Jesse RL, Warshaw MM, Valdes Jr R. National Academy of Clinical Biochemistry Standards of Laboratory Practice: Recommendations for the use of cardiac markers in coronary artery diseases. *Clin Chem* 1999; **45**: 1104-21
- 12 Panteghini M, Pagani F, Yeo K-TJ, *et al.* Evaluation of imprecision for cardiac troponin assays at low-range concentrations (on behalf of Committee on standardization of markers of cardiac damage of the IFCC). *Clin Chem* 2004; **50**: 327-32
- 13 Apple FS, Quist HE, Doyle PJ, *et al.* Plasma 99th percentile reference limits for cardiac troponin and creatine kinase MB mass for use with European Society of Cardiology/American College of Cardiology consensus recommendations. *Clin Chem* 2003; **49**: 1331-6
- 14 Thomas MA, Singh G, Williams EJ, *et al.* A review of troponin assay performance in Wales – can the same (method dependent) decision limits be used in different sites? *Ann Clin Biochem* 2005; **42**: 351-6
- 15 Collinson PO. Cardiac markers into the new millennium. *Ann Clin Biochem* 2000; **37**: 109-13
- 16 Panteghini M, Apple FS, Christenson RH, *et al.* Recommendations on use of biochemical markers of cardiac damage in acute coronary syndromes. *Scand J Clin Lab Invest* 1999; **59**(suppl 230): 103-12
- 17 Ross SM, Fraser CG. Biological variation of cardiac markers: analytical and clinical considerations. *Ann Clin Biochem* 1998; **35**: 80-4
- 18 Collinson PO. Early diagnosis of myocardial infarction: why measure cardiac enzymes? *J Clin Pathol* 1998; **51**: 2-4
- 19 Barsky AJ, Hochstrasser B, Coles NA, *et al.* Silent myocardial ischemia: is the person or the event silent? *JAMA* 1990; **264**: 1132-5
- 20 Hillis GS, Oliner C, O'Neil BJ, *et al.* Coronary artery disease in patients with chest pain who have low-risk clinical characteristics and negative cardiac troponin I. *Am J Emerg Med* 2001; **19**: 118-21
- 21 Tate J, Ward G. Interferences in immunoassay. *Clin Biochem Rev* 2004; **25**: 105-20
- 22 Kontos MC, Shah R, Fritz LM, *et al.* Implications of different cardiac troponin levels for clinical outcomes and prognosis of acute chest pain patients. *J Am Coll Cardiol* 2004; **43**: 958-65

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