Troponin
– when is an assay high sensitive?

Professor P. O. Collinson
MA MB BChir FRCPath FRCP edin MD FACB EurClin Chem
Consultant Chemical Pathologist and Professor of Cardiovascular Biomarkers,
Departments of Chemical Pathology and Cardiology, St George’s Hospital, London
High sensitivity troponin assays

• What makes an assay a high sensitivity assay
• How can you tell an assay is an hs assay
• Clinical advantages of hs Tn assays
• Issues with hs Tn assays
What makes an assay a high sensitivity assay
What makes an assay a high sensitivity assay

• A high sensitivity assay refers to the assays analytical characteristics, it does not mean a different form of troponin is being measured
• Total imprecision at the 99th percentile should be $\leq 10\%$
• The assay should detect at least 50% of healthy individuals above the LoD of the assay
• Results should be reported in ng/L

Apple and Collinson Clin Chem 2012;58:54-61
Assay performance to achieve a true 99\textsuperscript{th} centile

Diagnostic certainty

High

\[0\text{ ng/L}\]

\[5\text{ ng/L}\]

\[10\text{ ng/L}\]

\[15\text{ ng/L}\]

\[20\text{ ng/L}\]

\[25\text{ ng/L}\]

\[n\]

\[10\% \text{ CV}\]
Impact of assay performance on the 99th percentile - range of the 99th percentile

![Graph showing the impact of assay performance on the 99th percentile range. The graph compares 95% probability and 99% probability with CV (%). The x-axis represents %CV, and the y-axis represents the range of probabilities (52% and 26%). The graph includes data points and trend lines for both probabilities.](image-url)
Impact of 10% vs. 25% CV at the decision limit – shift in 99th centile

99th Percentile:
Tnl=0.063 at 10%CV
Tnl=0.070 at 25%CV

Apple et al Clin Chem 2005; 51 2198-2200
Impact of imprecision on reference change value (RCV) or delta change

- Utilises one tailed probability
  - +/- $1.65 \times \sqrt{2} \times \text{SD}$ or $2.33 \times \text{CVa}$. (95%)
  - +/- $2.33 \times \sqrt{2} \times \text{SD}$ or $3.30 \times \text{CVa}$ (99%)
Impact of analytical imprecision on the reference change value (RCV)

95% probability
99% probability

33%
59%
Short-term analytical and biological variation by hs-cTn assays.

<table>
<thead>
<tr>
<th></th>
<th>Abbott</th>
<th>Beckman</th>
<th>Roche (E170)</th>
<th>Siemens</th>
<th>Singulex</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV-A, %</td>
<td>13.8</td>
<td>14.5</td>
<td>7.8</td>
<td>13.0</td>
<td>8.3</td>
</tr>
<tr>
<td>CV-I, %</td>
<td>15.2</td>
<td>6.1</td>
<td>15.0</td>
<td>12.9</td>
<td>9.7</td>
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<tr>
<td>CV-G, %</td>
<td>70.5</td>
<td>34.8</td>
<td>NA</td>
<td>12.3</td>
<td>57</td>
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<tr>
<td>Index of individuality</td>
<td>0.22</td>
<td>0.46</td>
<td>NA</td>
<td>0.11</td>
<td>0.21</td>
</tr>
<tr>
<td>RCV, %</td>
<td>NA</td>
<td>NA</td>
<td>47.0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>RCV increase, %</td>
<td>69.3</td>
<td>63.8</td>
<td>NA</td>
<td>57.5</td>
<td>46.0</td>
</tr>
<tr>
<td>RCV decrease, %</td>
<td>−40.9</td>
<td>−38.9</td>
<td>NA</td>
<td>−36.5</td>
<td>−32</td>
</tr>
<tr>
<td>Within-individual mean, ng/L</td>
<td>3.5</td>
<td>4.9</td>
<td>NA</td>
<td>5.5</td>
<td>2.8</td>
</tr>
</tbody>
</table>
How can you tell an assay is an hs assay
Scorecard designations of cTn assays.

<table>
<thead>
<tr>
<th>Acceptance designation</th>
<th>Total imprecision at the 99th percentile, CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guideline acceptable</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Clinically usable</td>
<td>≥ 10 to ≤ 20</td>
</tr>
<tr>
<td>Not acceptable</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Assay designation</th>
<th>Measurable normal values below the 99th percentile, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 4 (third generation, hs)</td>
<td>≥ 95</td>
</tr>
<tr>
<td>Level 3 (second generation, hs)</td>
<td>75 to &lt; 95</td>
</tr>
<tr>
<td>Level 2 (first generation, hs)</td>
<td>50 to &lt; 75</td>
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<tr>
<td>Level 1 (contemporary)</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

Adapted from Apple Clin Chem 2009
Positive samples in a reference population

<table>
<thead>
<tr>
<th>Company/platform/assay</th>
<th>LOD</th>
<th>99th percentile ng/L</th>
<th>10% CV ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott Architect hs</td>
<td>1.9</td>
<td>26.2</td>
<td>4.7</td>
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<tr>
<td>Roche cTnT hs</td>
<td>5</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Beckman Access Accul+3</td>
<td>1</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Siemens Centaur Ultra</td>
<td>6</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Siemens Stratus CS</td>
<td>30</td>
<td>70</td>
<td>60</td>
</tr>
<tr>
<td>Siemens VISTA</td>
<td>15</td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>Siemens Dimension EXL</td>
<td>17</td>
<td>56</td>
<td>50</td>
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<tr>
<td>Mitsubishi Pathfast</td>
<td>8</td>
<td>29</td>
<td>14</td>
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<tr>
<td>Ortho Vitros ES</td>
<td>12</td>
<td>34</td>
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<tr>
<td>Abbott Architect</td>
<td>20</td>
<td>28</td>
<td>32</td>
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<tr>
<td>Beckmann Access AccuI</td>
<td>10</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Radiometer AQT90 cTnT</td>
<td>8</td>
<td>17</td>
<td>26</td>
</tr>
<tr>
<td>Radiometer AQT90 cTnI</td>
<td>9.5</td>
<td>23</td>
<td>39</td>
</tr>
</tbody>
</table>
Why shift to a hs assay

- Guideline recommended
- Assay performance
- Clinical utility
Evolution of Diagnostic Criteria for AMI using cTn

- **WHO**
  - Unstable Angina
  - Myocardial infarction

- **NACB**
  - Unstable Angina
  - MMD
  - Myocardial infarction

- **AHA/ESC**
  - Unstable Angina
  - Myocardial infarction

**Diagnostic limit for CKMB**

- 97.5 centile or LLD
- AMI Limit based on CKMB (ROC equivalent)

- 99th centile
Universal definition of myocardial infarction

Kristian Thygesen, Joseph S. Alpert and Harvey D. White on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction

Third universal definition of myocardial infarction

Kristian Thygesen, Joseph S. Alpert, Allan S. Jaffe, Maarten L. Simoons, Bernard R. Chaitman and Harvey D. White: the Writing Group on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction
Clinical advantages of hs Tn assays

- Role of other markers
- Speed of diagnosis
- Improved case detection
- Novel rule out strategies
Receiver operator characteristic curves for cardiac biomarkers for the diagnosis of acute myocardial infarction on admission.

Figure 1. Diagnostic Accuracy of Single Biomarker Testing for Acute Myocardial Infarction.

Shown are receiver-operating-characteristic curves and the corresponding areas under the curve for baseline measurements of troponin I with the sensitive assay (troponin I), troponin T, myoglobin, creatine kinase MB, and creatine kinase, according to the time of the onset of chest pain. Also shown are the sensitivity and specificity of these measures.
CK-MB

RIP

Myo

RIP

CK
Figure 2. Timing of conversion to positive troponin I (Tnl) in patients with myocardial injury. The percentage of patients with positive Tnl-Ultra (diamonds) and positive cardiac Tnl (cTnl; squares) is shown starting when the baseline specimen was obtained (ie, 0 hours) in a 24-hour period. By study design, all initial specimens for cTnl are negative.
Diagnostic Performance of Cardiac Troponin Assays at Presentation

Figure 1. Diagnostic Accuracy of Single Biomarker Testing for Acute Myocardial Infarction.

Shown are receiver-operating-characteristic curves and the corresponding areas under the curve for baseline measurements of troponin I with the sensitive assay (troponin I), troponin T, myoglobin, creatine kinase MB, and creatine kinase, according to the time of the onset of chest pain. Also shown are the sensitivity and specificity of these measures.
Receiver operating characteristic curve for biomarker measurements on admission for the diagnosis of acute myocardial infarction. cTnl CS 1, cTnl Stratus CS; cTnl B, cTnl Beckmann; cTnl S 1, cTnl Siemens; cTnT 1, cTnT; HFABP 1, heart fatty acid binding protein; Copeptin 1, copeptin.

Collinson P et al. Heart 2014;100:140-145
Design, Setting, and Patients  All consecutive patients admitted with suspected ACS to the Royal Infirmary of Edinburgh, Edinburgh, Scotland, before (n=1038; February 1-July 31, 2008, during the validation phase) and after (n=1054; February 1-July 31, 2009, during the implementation phase) lowering the threshold of detection for myocardial necrosis from 0.20 to 0.05 ng/mL with a sensitive troponin I assay were stratified into 3 groups (<0.05 ng/mL, 0.05-0.19 ng/mL, and ≥0.20 ng/mL). During the validation phase, only concentrations above the original diagnostic threshold of 0.20 ng/mL were reported to clinicians.

Main Outcome Measure  Event-free survival (recurrent MI and death) at 1 year in patients grouped by plasma troponin concentrations.
Figure. Survival Free From Death or Recurrent MI in Patients With Suspected Acute Coronary Syndrome Before (Validation Phase) and After (Implementation Phase) the Introduction of a Sensitive Troponin Assay

Validation phase
Peak troponin, ng/mL
- <0.05
- 0.05-0.19
- ≥0.20

Implementation phase
Peak troponin, ng/mL
- <0.05
- 0.05-0.19
- ≥0.20

Survival Free From Death or Recurrent MI, %

Log-rank P<.001
Proportion of men and women with diagnosis of type 1 myocardial infarction and type 2 myocardial infarction or myocardial injury using the contemporary troponin I assay (single threshold 50 ng/L) and high sensitivity troponin I assay (single threshold 26 ng/L, and sex specific threshold 34 ng/L for men and 16 ng/L for women).
Reduction of the impact of patient selection

• Shift to a hs troponin means a higher risk population can be ruled out
Rapid serial measurement of troponin supports early diagnosis

- Measurement of troponin on admission and at 1-2 hours
  - Contemporary assays with selection of low risk patients
  - High sensitivity assays – increase in risk groups that can be assessed
How will we use hs Troponin

• Rapid decision making based on existing strategies
• Rapid decision making with refinement of existing strategies
• Risk assessment plus different sampling strategies or decision points
Rapid decision making based on existing strategies
Patient presenting with chest pain? ACS

ECG indicates STEMI

ECG indicates NSTEMI

STEMI pathway
Troponin measurement on admission and 12-24 h to confirm diagnosis

Risk stratification

Initial cTn>99th percentile
significant change at 3 h or <99th percentile
significant change at 3 or 6 h

Initial cTn <99th percentile
no significant change at 3 or 6 h

NSTEMI

High-Intermediate risk

Low risk

Non ACS troponin elevation

High-Intermediate risk

Risk stratification

Assess to exclude other conditions

Normal/equivocal ECG

ETT or non-invasive imaging

Initial cTn <99th percentile
no significant change at 3 h

Low risk

Angiogram

Initial cTn>99th percentile
significant change at 3 h or <99th percentile
significant change at 3 or 6 h

ETT or non-invasive imaging

Non ACS troponin elevation

NSTEMI

Initial cTn >99th percentile
no significant change at 3 h

NSTEMI

Initial cTn <99th percentile
no significant change at 3 or 6 h

Initial cTn >99th percentile
no significant change at 3 or 6 h

Non ACS troponin elevation

Initial cTn >99th percentile
no significant change at 3 h

ETT or non-invasive imaging

Non ACS troponin elevation

Initial cTn >99th percentile
no significant change at 3 h

Initial cTn>99th percentile
significant change at 3 or 6 h

Initial cTn >99th percentile
no significant change
Patient presenting with chest pain? ACS

ECG indicates STEMI

STEMI pathway
Troponin measurement on admission and 6-24 h to confirm diagnosis

ECG indicates NSTEMI or is non-diagnostic

Risk stratification

cTn at 0,3 h

Initial cTn > 99th percentile
significant change at 3 h or
< 99th percentile
no significant change at 3 h

NSTEMI

High-Intermediate risk

Angiogram

Initial cTn < 99th percentile
no significant change at 3 h

Low risk

ETT or non-invasive imaging

Initial cTn > 99th percentile
no significant change at 3 h

Non ACS troponin elevation
Switch to hs troponin is accepted into UK recommendations

- [https://www.nice.org.uk/guidance/dg15](https://www.nice.org.uk/guidance/dg15)
- 1.1 The Elecsys Troponin T high-sensitive assay and ARCHITECT STAT High Sensitive Troponin-I assay are recommended as options for the early rule out of non-ST-segment-elevation myocardial infarction (NSTEMI) in people presenting to an emergency department with chest pain and suspected acute coronary syndrome.
- 1.2 The assays are recommended for use with 'early rule-out protocols', which typically include a blood sample for cardiac troponin I or T taken at initial assessment in an emergency department and a second blood sample taken after 3 hours. Laboratories should report absolute values and the upper reference limit should be set at the 99th percentile. Results should be interpreted along with clinical judgement and the results of clinical assessment. Healthcare professionals should take into account the pre-test probability of NSTEMI, the length of time since the suspected acute coronary syndrome, the possibility of chronically elevated troponin levels in some patients and that 99th percentile thresholds for troponin I and T may differ between sexes. When NSTEMI is not ruled out using an 'early rule-out protocol', further clinical assessment is required to determine whether a diagnosis of NSTEMI is appropriate.
Rapid decision making with refinement of existing strategies

- Impact of better imprecision in measurement
Aim of TRAPID-AMI:
to prospectively validate the hs-cTnT 1h-algorithm

Results

1282 Patients with chest pain

0h < 12 ng/L and Delta 1h < 3 ng/L
- Rule-out
  - 813 Patients (63.4 %)
    - NPV: 99.1 %

Others
- Observational zone
  - 285 Patients (22.2 %)
    - Prevalence of AMI: 22.5 %

0h ≥ 52 ng/L or Delta 1h ≥ 5 ng/L
- Rule-in
  - 184 Patients (14.4 %)
    - PPV: 77.2 %
Risk assessment plus different sampling strategies or decision points
hs Troponin done differently
- different decision thresholds

- Use of the Limit of Detection or Limit of Blank for Rule out with hs Troponin
  - It has been suggested that a cTn below the LoD measured on admission identifies a low risk group with a very high NPV
Meta analysis of studies looking at LOD for admission exclusion of AMI using hs assays

<table>
<thead>
<tr>
<th>Study</th>
<th>Prevalence (%)</th>
<th>TN/(TN+FN)</th>
<th>NPV</th>
<th>TP/(TP+FN)</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>RATPAC</td>
<td>8</td>
<td>605/612</td>
<td>0.998 [0.977, 0.995]</td>
<td>60/221</td>
<td>0.271 [0.224, 0.315]</td>
</tr>
<tr>
<td>ADOPT-Brisbane</td>
<td>8.1</td>
<td>269/270</td>
<td>0.996 [0.978, 1.000]</td>
<td>66/562</td>
<td>0.117 [0.106, 0.125]</td>
</tr>
<tr>
<td>Manchester</td>
<td>18.5</td>
<td>232/232</td>
<td>1.000 [0.983, 1.000]</td>
<td>130/471</td>
<td>0.276 [0.258, 0.291]</td>
</tr>
<tr>
<td>APACE</td>
<td>20.4</td>
<td>627/628</td>
<td>0.998 [0.991, 1.000]</td>
<td>576/2204</td>
<td>0.261 [0.255, 0.267]</td>
</tr>
<tr>
<td>ADOPT-Christchurch</td>
<td>23.4</td>
<td>122/123</td>
<td>0.992 [0.949, 1.000]</td>
<td>251/953</td>
<td>0.263 [0.255, 0.270]</td>
</tr>
<tr>
<td>FAST and FASTER</td>
<td>36.4</td>
<td>108/115</td>
<td>0.939 [0.868, 0.978]</td>
<td>138/283</td>
<td>0.488 [0.449, 0.524]</td>
</tr>
</tbody>
</table>

Summary estimates: NPV = 0.992 [0.952, 0.999]; PPV = 0.253 [0.203, 0.338]

<table>
<thead>
<tr>
<th>Study</th>
<th>TP/(TP+FN)</th>
<th>Sensitivity</th>
<th>TN/(TN+FP)</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>RATPAC</td>
<td>60/67</td>
<td>0.886 [0.797, 0.957]</td>
<td>605/67</td>
<td>0.790 [0.759, 0.818]</td>
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<tr>
<td>ADOPT-Brisbane</td>
<td>66/67</td>
<td>0.985 [0.920, 1.000]</td>
<td>269/765</td>
<td>0.352 [0.318, 0.387]</td>
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<tr>
<td>Manchester</td>
<td>130/130</td>
<td>1.000 [0.972, 1.000]</td>
<td>232/573</td>
<td>0.405 [0.364, 0.446]</td>
</tr>
<tr>
<td>APACE</td>
<td>576/577</td>
<td>0.998 [0.990, 1.000]</td>
<td>627/2254</td>
<td>0.278 [0.260, 0.297]</td>
</tr>
<tr>
<td>ADOPT-Christchurch</td>
<td>251/252</td>
<td>0.996 [0.978, 1.000]</td>
<td>122/924</td>
<td>0.148 [0.124, 0.174]</td>
</tr>
<tr>
<td>FAST and FASTER</td>
<td>138/145</td>
<td>0.952 [0.903, 0.980]</td>
<td>108/253</td>
<td>0.427 [0.365, 0.490]</td>
</tr>
</tbody>
</table>

Summary estimates: Sensitivity = 0.985 [0.947, 0.996]; Specificity = 0.609 [0.409, 0.778]
Summary receiver operating characteristics plot of sensitivity and specificity for cut-off value of either 3 ng/L or 5 ng/L.

Zhivko Zhelev et al. BMJ 2015;350:bmj.h15
What is the optimal threshold to rule out myocardial infarction?

Admission hs-cTnI < 5ng/L rules out composite outcome of death or MI within 30 days correctly in 99.6% of patients

52% of patients with suspected acute coronary syndrome have hs-cTnI concentrations < 5ng/L on presentation

n=4,886
Issues with hs Tn assays
Assay problems

- TnI
- TnC
- Heparin
- Phosphorylated Ser22 and Ser23
- Sites of protease cleavage
- Anti-TnI autoantibodies
Defining normality

• 1392 individuals randomly selected from the general population invited by a letter to participate, 734 (52.7%) responded and therefore enrolled in the study.
• Detailed information regarding the participants was collected by a questionnaire.
• Investigations
  – Heart rate and blood pressure measurement (the mean of 2 readings)
  – Spirometry
  – ECG
  – Echocardiography
  – Venesection for fasting plasma glucose, creatinine, and cardiac troponin measurements.

Fig. 1. Frequency distribution histograms and 99th percentile values (claimed and derived from the data) for the 3 assays with no patient selection (upper row), screening by questionnaire alone (middle row), and selection by questionnaire, blood pressure, eGFR, and imaging results as described in the methods section. All measurements are in nanograms per liter.
Unadjusted Nelson-Aalen Curves for All-Cause and Cardiovascular Mortality

de Lemos, J. A. et al. JAMA 2010;304:2503-2512
Forest plot of primary studies evaluating abnormally elevated troponin T and all-cause mortality in patients with renal dysfunction
Age-Standardized Rates of Death from Any Cause (Panel A), Cardiovascular Events (Panel B) according to the Estimated GFR among 1,120,295 Ambulatory Adults

Discharge of low risk patients is safe and feasible

- A troponin within the reference interval has a powerful negative predictive value
- An elevated troponin indicates an underlying pathophysiological problem
Acute chest pain or other symptoms suggestive of myocardial ischemia

Clinical history*

ECG*
cTn*

No myocardial ischemia or injury
Consider other diagnosis

Evidence of myocardial ischemia and/or injury

Coronary angiography* & other imaging techniques

Type 1 AMI
Unstable Angina
Secondary Myocardial Injury – acute (Type 3-5 AMI)
Non-ischaemic myocardial injury – acute
Myocardial injury - chronic

ACS

Collinson and Lindahl Heart 2015
Questions