Normal Plasma Levels of Cardiac Troponin IMeasured by the High-Sensitivity Cardiac Troponin I Access Prototype Assay and the Impact on the Diagnosis of Myocardial Ischemia

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Objectives
This study sought to evaluate the analytical and clinical performance of the novel hypersensitive cardiac troponin I (cTnI) prototype assay from Beckman Coulter (Fullerton, California).

Background
Studies on patients with acute coronary syndromes and on seemingly healthy subjects have shown that even very minor elevations of cardiac troponins are associated with an increased risk of death. However, the normal plasma levels of cardiac troponins are still not known.

Methods
cTnI plasma levels were measured in 542 healthy subjects, 319 men (age 59.9 ± 11.8 years) and 213 women (age 59.8 ± 13.1 years), and in 1,503 randomly selected patients of the GUSTO IV (Global Utilization of Strategies To open Occluded arteries IV) cohort with unstable angina and non-ST-segment elevation myocardial infarctions (MIs).

Results
The cTnI levels at 10% coefficient of variation and 20% coefficient of variation imprecision were 0.0033 and 0.0016 μg/l, respectively. The cTnI levels were measurable in >95% of the healthy subjects. The median level of healthy subjects <60 years of age was 0.0032 μg/l (range 0.0011 to 0.0079 μg/l) with the 99th percentile being 0.010 μg/l. No sex differences were observed. A receiver-operator characteristic curve analysis showed an optimal discrimination between healthy subjects and patients at 0.0064 μg/l with a sensitivity of 84.8% (95% confidence interval: 82.8% to 86.6%) and specificity of 89.7% (95% confidence interval: 86.8% to 92.2%). Outcomes as to death and/or MI were significantly different at this level (p < 0.01) in the GUSTO IV cohort.

Conclusions
The novel high-sensitivity cTnI prototype assay from Beckman Coulter allows for the first time the measurement of cTnI levels in almost all healthy subjects. Our data indicate that the assay may be a powerful aid in the diagnosis and outcome prediction of patients with suspected myocardial ischemia and question any definition of myocardial infarction. (J Am Coll Cardiol 2009;54:1165–72) © 2009 by the American College of Cardiology Foundation

The assay of blood levels of cardiac troponins has become the recommended procedure for the detection of cardiac injury in patients with the acute coronary syndrome (ACS) and acute myocardial infarction (AMI) (1). Elevated levels, however, may also be seen in subjects without apparent signs of cardiovascular disease and are in these cases important predictors of future episodes of AMI and/or premature death in cardiovascular disease (2,3). As recommended by the consensus documents, cardiac troponins should be measurable at the 99th percentile of the upper reference limit (URL) in healthy subjects with a maximum imprecision of 10% coefficient of variation (CV) (4,5), a requirement currently only met by a few available assays. In previous reports we showed that the 99th percentile is age dependent, because elderly subjects in many cases had signs
of elevated levels above those of younger subjects, suggesting ongoing subclinical processes in a portion of seemingly healthy subjects (6). Thus, the proper definition of the 99th percentile URL is extremely difficult and very much dependent on the study cohort.

Recent developments and improvements of cardiac troponin assays have clearly indicated that subjects with even very minor elevations of cardiac troponins are at increased risk of premature MI and/or death (3,7–9). Thus, the more sensitive the assay is, the more patients are identified at risk. The open question therefore remains, “How sensitive should a cardiac troponin assay be?” This question will only be answered with a cardiac troponin assay that is able to measure any elevations with acceptable imprecision, and to identify such elevations it is necessary to know what the normal levels of cardiac troponins are. In this report we show the results of such a development, as the recent prototype cardiac troponin I (cTnI) assay by Beckman Coulter is able to identify troponin I levels in virtually all healthy subjects, and this assay allows us to define the discrimination between healthy subjects and those with ACS with high accuracy.

The study on healthy subjects consisted of 537,388 men (median age 62 years, range 28 to 76 years) and 213 women (median age 64 years, range 29 to 76 years), of which 42 subjects were above the age of 75 years; 384 of the subjects were included in the SWISCH (Sweden Women and Men and Ischemic Heart Disease) study during 2000 to 2001 and 153 were included in a cohort of healthy employees. Employees were included if found healthy after a general physical examination by a physician, a normal electrocardiogram at rest, and a questionnaire-based survey of previous and present disease that did not indicate any health problems. The SWISCH study was a case-control study of risk factors for coronary artery disease in older men and women (12). In this study, subjects were randomly recruited from the population using the registry and matched for age and sex with patients with unstable coronary artery disease included at 6 hospitals in the FRISC II (Fragmin and Fast Revascularisation during Instability in Coronary artery disease) trial during 1996 to 1998. Details of these studies were published previously (13,14). The FRISC II and GUSTO IV trial populations were strikingly similar (15,16).

The imprecision profile of the assay was estimated from the analysis of duplicate samples of 306 patients admitted to the emergency department with suspicion of cardiac disease. The samples were leftover plasma and were analyzed without knowledge of the identity or diagnosis of the patients. The procedure was approved by the local ethics committee.

Methods

The cTnI assay was a prototype assay supplied by Beckman Coulter and was run on the Access instrument (Beckman Coulter, Fullerton, California) (17). Levels >10 μg/l were not further analyzed but assigned the value of 10 μg/l. The AccuTnI assay was run on the Access instrument according to instructions of the manufacturer.

Statistics. Passing-Bablok regression analysis, Mann-Whitney nonparametric test for group comparisons, chi-square test, and receiver-operator characteristic (ROC) curve analysis were used. The imprecision profile of the Access high-sensitivity troponin I assay was estimated using the Sadler and Smith method (18). In brief, a 3-parameter relationship between variance of the replicate measurements of cTnI with the high-sensitivity cTnI assay and their mean concentrations was estimated using unweighted nonlinear least-squares regression. Outliers were identified as samples that were either 3 times higher or 3 times lower than the interquartile levels of the whole reference population (19). In Passing-Bablok regression analysis, only results <10 μg/l were included. All calculations were made by the statistical software Medcalc version 9 (Mariakerke, Belgium) and Statistica for Windows version 8 (Tulsa, Oklahoma).
Results

The imprecision profile, based on CVs of assaying duplicate samples of 306 consecutive patients with suspicion of cardiac disease, showed an estimated 10% CV at 0.0033 µg/l and an estimated 20% CV at 0.0016 µg/l (Fig. 1). Overall the high-sensitivity cTnI assay was significantly correlated to the AccuTnI assay, $r^2 = 0.88$ ($p < 0.001$) ($n = 1,343$ GUSTO IV samples). A Passing-Bablok regression analysis showed high-sensitivity cTnI $= -0.01566 + 0.6372$ AccuTnI with a significant deviation from linearity.

The cTnI levels in the cohort of seemingly healthy subjects are shown in Figure 2 and indicate that 93% of all samples had levels above the 20% CV cutoff of 0.0016 µg/l and 50% above the 10% cutoff of 0.0033 µg/l. The majority of results formed a bell-shaped distribution, with 93% of the results being $< 0.010$ µg/l. With all results from the reference samples included, the 99th and 97.5th percentiles were 0.064 and 0.029 µg/l, respectively. After outlier exclusions the 97.5th and 99th percentiles were found at 0.0110 and 0.0144 µg/l, respectively. For the cohort $\geq 60$

<table>
<thead>
<tr>
<th>% CV</th>
<th>Concentration µg/l</th>
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<tbody>
<tr>
<td>10</td>
<td>0.0033</td>
</tr>
<tr>
<td>20</td>
<td>0.0016</td>
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</tbody>
</table>

Figure 1  The Imprecision Profile of the High-Sensitivity cTnI Access Assay

The inset shows the profile with the x-axis truncated at 0.030 µg/l and also the estimated 10% and 20% coefficients of variation (CVs). The 95% confidence interval of the fitted line is shown. cTnI = cardiac troponin I.

Figure 2  The Distribution Profile of cTnI in Healthy Subjects

(A) The distribution of cardiac troponin I (cTnI) levels in the seemingly healthy population. The x-axis was truncated at 0.030 µg/l. (B) All results from the seemingly healthy subjects divided by sex. No statistical differences were found between sexes.
years of age, the 97.5th and 99th percentiles were 0.012 and 0.019 µg/l, respectively, and for the cohort <60 years of age were 0.009 and 0.010 µg/l, respectively (Fig. 3). No sex differences were observed in either of these 2 age cohorts (Fig. 3). For the whole cohort, no age difference was found, whereas among those subjects who had levels ≥0.010 µg/l, 75% were older than 60 years, as compared with 56% in the cohort with levels <0.010 µg/l (p = 0.03).

In the analysis of a subset of the GUSTO IV cohort, the results showed significantly elevated levels as compared with the healthy subjects (p < 0.001) (Fig. 4). Twenty percent of the GUSTO IV subjects had levels below 0.010 µg/l. An ROC curve analysis including all results from the healthy cohort showed an area under the curve (AUC) of 0.942 (95% confidence interval [CI]: 0.931 to 0.952) with a sensitivity and specificity in the discrimination between ACS and healthy subjects of 88.4% (95% CI: 86.6% to 89.9%) and 85.0% (95% CI: 81.7% to 87.9%), respectively, at the optimal cutoff of 0.0064 µg/l (Fig. 5). After outlier exclusion, the AUC increased marginally to 0.948 (not significant). Adopting the 99th percentile cutoffs after outlier exclusion, that is, 0.01 µg/l and 0.019 µg/l for those <60 years old and for the whole cohort, respectively, the specificities increased substantially to 95.2% and 99.1%, respectively (Table 1).

The GUSTO IV study protocol does not allow the distinction between patients with non–ST-segment elevation myocardial infarction (MI) and unstable angina. As a surrogate marker for this distinction, we therefore used cTnT with a cutoff of 0.03 µg/l, corresponding to the 10% CV level for the third-generation TnT assay on an Elecsys

Figure 3 The 99th and 97.5th Percentile Levels of cTnI in Seemingly Healthy Subjects

(A) Data from subjects <60 years of age and divided by sex. (B) Data from subjects ≥60 years of age and divided by sex. No statistical differences between sexes were found in either of the 2 cohorts. The percentile levels were defined after exclusion of outliers. In subjects <60 years of age results ≥0.012 µg/l and in subjects ≥60 years of age results ≥0.028 µg/l were defined as outliers. Abbreviations as in Figure 1.

Figure 4 cTnI Levels in Healthy Subjects and Patients With ACS

The comparison of cTnI levels in seemingly healthy subjects and in patients with the acute coronary syndrome (GUSTO IV [Global Utilization of Strategies To open Occluded arteries IV]). Results above 10 µg/l were assigned 10 µg/l. The difference in cTnI levels between the cohorts was highly significant (p < 0.0001). ACS = acute coronary syndrome; other abbreviations as in Figure 1.
<0.03 µg/l (p < 0.001). The cTnI levels in the subset with elevated cTnT levels were on average more than 50-fold increased. Figure 7 shows the ROC curve analysis of the 2 subsets and the diagnostic distinction from healthy subjects. In the subset with nonelevated cTnT, the AUC was 0.848 (95% CI: 0.825 to 0.869). The optimal cutoff was 0.0046 µg/l, with a corresponding sensitivity and specificity of 79.5% (95% CI: 75.8% to 82.8%) and 76.0% (95% CI: 72.2% to 79.6%), respectively. The positive likelihood ratio was 3.31 (95% CI: 3.1 to 3.5), and the negative likelihood ratio was 0.27 (95% CI: 0.2 to 0.3). In the subset with elevated cTnT, the AUC was 0.996 (95% CI: 0.991 to 0.999). The optimal cutoff was 0.0164 µg/l with a corresponding sensitivity and specificity of 98.7% (95% CI: 97.8% to 99.4%) and 96.3% (95% CI: 94.4% to 97.7%), respectively. The positive likelihood ratio was 26.2 (95% CI: 26.3 to 27.2), and the negative likelihood ratio was 0.013 (95% CI: 0.006 to 0.03).

Patient outcomes regarding death in 1 year or the composite end point of death and/or MI within 30 days is shown in Table 2. In total, 110 deaths in 1 year and 106 deaths and/or MI were observed in the cohort. No events were seen below the median levels for healthy subjects and 4 and 6 events, respectively, below the optimal cutoff of 0.0064 µg/l. The difference in cTnI levels between the cohorts was highly significant (p < 0.001; analysis of variance) as were the differences between healthy subjects and either of the 2 GUSTO IV subsets (p < 0.0001). Abbreviations as in Figures 1 and 4.

**Discussion**

The prototype cTnI assay used in this report was shown to have a sensitivity sufficient to measure cTnI levels in almost all healthy subjects. Thus, for the first time we have been able to define normal levels and have shown that they essentially follow a Gaussian distribution. By this assay we

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**Table 1**

<table>
<thead>
<tr>
<th>Cutoff Level, µg/l</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>+LR</th>
<th>−LR</th>
</tr>
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<tbody>
<tr>
<td>0.0032 (median)</td>
<td>98.3 (97.5–99.0)</td>
<td>50.2 (45.8–54.5)</td>
<td>1.97</td>
<td>0.34</td>
</tr>
<tr>
<td>0.0046 (ROC curve)</td>
<td>84.8 (82.8–86.6)</td>
<td>89.7 (86.8–92.2)</td>
<td>8.26</td>
<td>0.17</td>
</tr>
<tr>
<td>0.009 (97.5th percentile, age &lt;60 yrs)</td>
<td>78.9 (76.7–81.0)</td>
<td>93.9 (91.5–95.8)</td>
<td>13.0</td>
<td>0.22</td>
</tr>
<tr>
<td>0.010 (99th percentile, age &lt;60 yrs)</td>
<td>77.5 (75.3–79.7)</td>
<td>95.2 (93.1–96.9)</td>
<td>16.3</td>
<td>0.24</td>
</tr>
<tr>
<td>0.012 (97.5th percentile, age ≥60 yrs)</td>
<td>75.3 (73.0–77.5)</td>
<td>97.5 (95.8–98.7)</td>
<td>30.5</td>
<td>0.25</td>
</tr>
<tr>
<td>0.019 (99th percentile, age ≥60 yrs)</td>
<td>70.5 (68.1–72.9)</td>
<td>99.1 (97.8–99.7)</td>
<td>74.2</td>
<td>0.30</td>
</tr>
</tbody>
</table>

−LR = negative likelihood ratio; +LR = positive likelihood ratio; CI = confidence interval; cTnI = cardiac troponin I; GUSTO IV = Global Utilization of Strategies To open Occluded arteries IV; ROC = receiver-operator characteristic.
also have shown that men and women have equal levels and that the levels were independent of age. The only apparent age and sex differences were related to the fact that outliers were overrepresented in the elderly cohort and in particular among men. This may be a reflection of subclinical disease in some subjects with chronic myocardial injury and is not unexpected considering the fairly advanced age of the reference population. Slight cTnI elevations were previously found in a cohort of seemingly healthy 70-year-old men and were shown to be related to premature death in cardiovascular disease (3). We also found that subjects with documented signs and symptoms of myocardial ischemia, also when not fulfilling the current definition of AMI (i.e., cTnT >0.03 µg/l), had on average significantly higher levels than seemingly healthy subjects and that the event rates regarding death and MI were related to the cTnI levels even at very low levels.

We defined the functional sensitivity of the cTnI by means of the imprecision profile derived from the CVs of duplicate measurements of consecutive samples of patients with suspicion of cardiac disease obtained from our emergency department. The 10% and 20% CVs from these results are extrapolated estimates because these CVs were not exceeded at any cTnI concentration. However, the reasonably correct estimates of the levels obtained were supported by the distribution profile of the levels of the reference samples because these also showed a harmonious Gaussian distribution at the lower end of the concentrations. Our results also highlight the difficulties in the establishment of clinically useful cutoffs. When we included all results from our reference samples, the 99th and 97.5th percentiles were 0.064 and 0.029 µg/l, respectively. A look at the distribution graph, however, clearly indicated that these levels were governed by results not belonging to the distribution of the majority of results, which belonged to the Gaussian distribution. Therefore, we applied an outlier algorithm (19), which eliminated all results exceeding 3× the upper quartile level. After such correction, the 99th and 97.5th percentiles were 0.022 and 0.012 µg/l, respectively, and it was quite apparent that the distribution of the results

| Table 2 | Outcomes in Patients With the Acute Coronary Syndrome (GUSTO IV) at Different Cutoff Levels of the High-Sensitivity cTnI Access Assay |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Cutoff Level, µg/l | Death or Myocardial Infarction at 30 Days | Death at 1 Year |
| Below | Equal to or Above | p Value* | Below | Equal to or Above | p Value* |
| 0.003 (median and 10% CV) | 0% (0) | 7.9% (110) | 0.12 | 0% (0) | 7.6% (106) | 0.14 |
| 0.006 (ROC) | 2.0% (4) | 8.6% (106) | 0.001 | 3.0% (6) | 8.1% (100) | 0.01 |
| 0.009 (97.5th percentile, age <60 yrs) | 2.7% (8) | 9.1% (102) | <0.001 | 2.7% (8) | 8.7% (98) | <0.001 |
| 0.010 (99th percentile, age <60 yrs) | 3.1% (10) | 9.1% (100) | <0.001 | 2.8% (9) | 8.8% (97) | <0.001 |
| 0.012 (97.5th percentile, age ≥60 yrs) | 3.4% (12) | 9.1% (98) | <0.001 | 2.6% (9) | 9.0% (97) | <0.001 |
| 0.019 (99th percentile, age ≥60 yrs) | 4.5% (19) | 9.1% (91) | 0.004 | 4.5% (19) | 8.7% (87) | 0.007 |

*Chi-square analysis. CV = coefficient of variation; other abbreviations as in Table 1.
of the younger cohort, those <60 years of age, adapted to a harmonious Gaussian distribution, whereas the results of the older cohort still had a tail upward not included in the Gaussian distribution. In previous studies we showed that the application of the 99th percentile for the cohort of younger subjects actually identified more subjects at risk of premature death and/or MI (3,7). A similar approach in this study would mean a 99th percentile URL of 0.010 µg/l, and as shown this was actually the case. The intriguing finding in this study, however, is that we found significant differences in event rates even at lower levels.

One of the most spectacular findings in this study was the large difference between the results of the healthy cohort and the results of the GUSTO IV cohort. These results indicate that the vast majority of subjects included in the GUSTO IV study had leakage of cTnT reflecting injury to the myocardium likely as a consequence of acute ischemia in most cases. One important question, therefore, is whether normal levels of cTnI, as measured by the hypersensitive Access cTnI assay, can be used to exclude, rule out, myocardial ischemia. The ROC curve analysis showed the optimal discrimination of health and disease at the level of 0.0064 µg/l. At this level, the negative predictive value was 68.5%, but to achieve negative predictive values of more than 90% the cTnI levels should be below the median levels of the healthy subjects, that is, 0.0032 µg/l. Indeed, below this level we had no adverse outcomes regarding death within 1 year or death/MI within 30 days among the GUSTO IV subjects. The above calculations were made on the assumption of accurate inclusions of all subjects among the healthy and diseased cohorts, which may not be the case. In a previous randomized clinical trial including patients with a clinical diagnosis of unstable angina, 14% of the patients included in the trial had no significant luminal diameter stenosis of a major coronary artery (20), and in another ACS trial (21), 32% of those without detectable troponin T had no significant coronary stenosis. Hence, it is likely that a small proportion of patients included in the GUSTO IV trial had other causes of their chest pain at inclusion than true myocardial ischemia. Indeed, when we used cTnT levels as surrogate markers of non–ST-segment elevation MI and unstable angina, the distinction between MI and healthy subjects was very accurate, whereas the distinction between unstable angina and healthy subjects was less accurate, with sensitivities and specificities in the range of 75% to 80%. Therefore, further studies in carefully characterized patient materials are needed to establish the true sensitivity and specificity and to accurately define decision limits. However, the present results suggest that it might be possible to define such rule-out limits, and this would obviously have a very important impact on the clinical management of patients with suspected ACS. Slightly elevated levels of cTnI, however, are found, aside from ACS in many other disorders affecting the heart directly or indirectly (22,23). Therefore, in the future management of our patients with the aid of these hypersensitive cardiac troponin assays, we may need to define new decision limits and criteria for the diagnosis of acute ischemic diseases of the myocardium and for prediction of future events. The current consensus criteria suggest a rise and fall above the 99th percentile URL in cardiac troponins in addition to other symptoms and signs of ischemia as the diagnostic criteria for AMI (1). With the hypersensitive cardiac troponin assays and with the lowering of the 99th percentile further, it is predicted that such changes will be seen much more frequently in patients without classical AMI caused by plaque rupture with a superimposed thrombus in the coronary arteries, and that this will prompt the clinician to be more active in defining the specific cause of the elevation in each case. Therefore, can we define criteria and decision limits that will guide the clinician to take appropriate action without jeopardizing the safety of our patients? When it comes to prediction, the situation seems slightly less complicated because this and other studies have shown that even minor elevations of cardiac troponins are associated with unfavorable long-term outcome and an increased risk of premature death and/or MI (3,7,8). Thus, any elevations above the low 99th percentile are important signs, and the situation seems almost dichotomous. Highly sensitive troponin assays, therefore, might also be useful in the screening situation of populations at risk. With the current knowledge we may now adopt conventional criteria for the definition of what are normal or abnormal levels of cTnI, that is, use the 97.5th percentiles instead of the 99th percentiles because of the inherent difficulties of accurate definition of the latter. In addition to this, we may define levels below or above these levels to meet specific clinical needs, but as indicated previously, this will have to await further studies.

Conclusions

The hypersensitive cTnI assay from Beckman Coulter allows us to define normal plasma levels of cTnI. This information is novel and very important for our understanding of how to optimize the clinical utility of cTnI measurements in the diagnosis and outcome prediction of patients with cardiovascular disease. Our data also suggest that the hypersensitive cTnI assay may be a means to rule out patients with suspected ischemia of the myocardium and an unfavorable outcome.

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Key Words: troponin I • unstable angina • myocardial infarction.