Metaanalysis in Clinical Chemistry: Validation of Cardiac Troponin T as a Marker for Ischemic Heart Diseases

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Metaanalysis is a method that incorporates the pooling of previously published results to produce more statistically significant results. We used metaanalysis to examine the role of a new cardiac marker, cardiac troponin T (cTnT), in patients with ischemic heart disease. Metaanalysis of six articles and one abstract on cTnT showed that this marker was just as sensitive as creatine kinase MB isoenzyme (CK-MB) for the retrospective diagnosis of acute myocardial infarction (AMI) 12–48 h after onset but less specific. Most of these articles showed that cTnT was increased in non-AMI patients with unstable angina pectoris. In a metaanalysis of four papers, two abstracts, a letter, and an unpublished manuscript, we examined the prognostic role of cTnT in non-AMI cardiac patients. For an unfavorable endpoint defined as cardiac death, AMI, or the need for coronary artery revascularization, the results demonstrated that abnormal concentrations of cTnT were associated with a higher risk for a poor outcome than were normal concentrations of cTnT. We also compared cTnT with CK-MB for risk stratification. Metaanalysis will become an increasingly important tool for evaluating new tests as they become available.

**Indexing Terms:** acute myocardial infarction/unstable angina/CK-MB compared

Clinical trials of new therapeutic regimens or diagnostic tests are mainstays in clinical and laboratory medicine. In cases of a clearcut advantage between an old and a new method, e.g., the thymol turbidity test vs serum alanine aminotransferase for detecting hepatic parenchymal damage, valid statistical conclusions can be made with a relatively small clinical study. For comparisons between drugs or laboratory tests that have similar performances, larger numbers of subjects may be necessary to reach statistically valid conclusions. If the prevalence of the disease in question is low, however, such studies may be too prohibitive for a single study site to enroll sufficient numbers of subjects in a reasonable amount of time. Multicenter trials are used to increase the statistical power, but these trials can be very expensive and are less likely than a single site to employ uniform practices.

The traditional approach towards combining individual studies of a given subject is the comprehensive review article. These articles, usually invited and written by “experts” in a given area, provide narrative summaries and criticisms of existing knowledge. Metaanalysis is a statistical technique used to combine and evaluate published results of clinical studies performed under similar conditions (1–4). By increasing the subject database, metaanalysis can be used to resolve differences in conclusions made in published reports. In addition, new questions not posed by individual studies can be raised and addressed.

The majority of published reports on the applications of metaanalysis have involved trials of new drugs or therapeutic regimens, e.g., many trials comparing different treatments for patients with acute myocardial infarction (AMI). 2 2 Stampfer et al. (5) used metaanalysis to show that streptokinase reduced the mortality of AMI patients by 20%.

A specialized form of metaanalysis is the cumulative metaanalysis (6). In this approach, reports included in the analysis are listed chronologically. A new metaanalysis is performed each time that a new clinical trial meeting the inclusion criteria is published. In this manner, one can report the year when the combined results of multiple trials first achieve a given level of statistical significance. For example, Antman et al. (7) showed that the use of oral β-blockers was first statistically demonstrated to be effective in the secondary prevention of myocardial infarction in 1977, after enrollment of 3522 total patients. Subsequent published studies further verified this conclusion—a finding suggesting that many of these later trials were unnecessary.

Compared with metaanalyses of therapeutic procedures, only a few published metaanalyses have been performed for diagnostic tests used in a clinical laboratory. Ried et al. (8) combined 14 studies to show that implementation of a therapeutic drug monitoring program reduced the incidence of toxic drug reactions for patients given digoxin, theophylline, phenytoin, and aminoglycosides. Offerings and Benbasset (9) examined reports of urinary red blood cell shape in the diagnosis of glomerular and postglomerular hematuria. Hurlbut et al. (10) combined 51 papers to show that a positive dipstick result for either nitrite or leukocyte esterase or both was accurate for diagnosis urinary tract infections. Phillips (11) applied metaanalysis to report sensitivity and specificity for enzyme immunosorbent assays of human immunodeficiency virus. Recently, Irwig et al. (12) established guidelines for metaanalysis for evaluating diagnostic tests, outlining the need for a mission statement and showing how to retrieve

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2 Nonstandard abbreviations: AMI, acute myocardial infarction; cTnT, cardiac troponin T; CK-MB, creatine kinase MB isoenzyme; CI, confidence interval; UA, unstable angina; TPR, true-positive rate; FPR, false-positive rate; and SROC, summary receiver-operating characteristic.
relevant literature, extract data, estimate diagnostic accuracy, and assess variables and characteristics that affect accuracy. These guidelines also described how data on clinical sensitivity and specificity from various published reports could be displayed by using summary receiver-operating characteristic (SROC) curves.

We used metaanalysis to examine the use of cardiac troponin T (cTnT) for patients with coronary artery diseases. We compared the diagnostic accuracy of cTnT with that of creatine kinase MB isoenzyme (CK-MB) for retrospective AMI diagnosis (12–48 h after onset). We also wanted to determine whether cTnT could be used as a prognostic marker for patients with unstable angina (UA). The clinical outcomes studied were death, nonfatal AMI, and the need for coronary artery revascularization.

**Materials and Methods**

We performed three separate metaanalyses: a comparison of CK-MB (mass assays) with cTnT in AMI patients, and the prognostic role of cTnT for cardiac death and nonfatal AMI and the need for revascularization in non-AMI patients. In all analyses, only papers written in English were reviewed. We also excluded review articles because they did not include any primary data previously unpublished. The studies under consideration were reviewed by both authors, with disagreements between us being settled by a third individual.

**cTnT for Retrospective AMI Diagnosis**

We performed a MEDLINE search of articles published from January 1985 to February 1995, using the combined keywords troponin and acute myocardial infarction. Pre-1985 literature was not considered because cTnT assays were not available then. In all, 37 articles were identified. We also searched published abstracts from national meetings and attempted to obtain additional information from the authors of pertinent abstracts so that their data could be considered in the metaanalysis. The inclusion criteria were: (a) data available for clinical sensitivity and specificity for both cTnT and CK-MB, and (b) time of blood collection specified as being within 12–48 h after admission or onset of chest pain. Although we did not exclude them from the literature search, we did not include the results of four papers involving cardiac troponin I, there currently being no commercially available assays for this marker in the US (all four papers used experimental assays). Five articles on cTnT were excluded because one did not contain comparative CK-MB data, two did not include specificity data for CK-MB, one was specifically aimed at early diagnosis (<12 h after onset of chest pain), and one compared cTnT with an immunoinhibition assay of CK-MB. This last paper was excluded because of the well-documented poor specificity of immunoinhibition (13). In summary, we subsequently selected a total of six articles for the metaanalysis (14–19). In addition, we included one abstract for which the primary data were made available (20). In this abstract and in the paper by Wu et al. (17), the cTnT cutoff concentration was 0.1 μg/L. In all of the others reports, the cutoff concentration was 0.2 μg/L. cTnT was assayed in each report by using the Cardiac assay (Boehringer Mannheim, Indianapolis, IN) and the ES 300 analyzer (Boehringer Mannheim).

Assays used for CK-MB in these studies included NovoClone (Novo Biolabs, Dako, Denmark), Tandem-E (Hybritech, San Diego, CA), Stratus II (Baxter Healthcare, Miami, FL), IMx (Abbott Labs., Abbott Park, IL), and Magic Lite (Ciba-Corning, Walpole, MA). Because of the lack of standardization for CK-MB mass assays, different cutoff concentrations were used for these mass assays. In some cases, a relative index was also included (%CK-MB/total CK). No attempt was made to correct results to a single cutoff concentration for either cTnT or CK-MB. The lack of a standard assay may have degraded the collective performance for CK-MB for diagnosis of AMI.

**cTnT in Non-AMI Patients**

We also performed a MEDLINE search covering the period from January 1990 to February 1995, using the combined keywords troponin and unstable angina. This identified a total of 16 papers. We also included in the metaanalysis two abstracts and one unpublished manuscript made available to us. In none of these studies were cTnT results used in the prospective management of the subjects studied. For the metaanalysis based on using death and nonfatal AMI as the outcome, we selected three papers (16, 21, 22), one letter to the editor (23), one abstract (24), and one manuscript (Stubbs F, Collinson P, Moseley D, Oakes M, Greenwood TW, Noble MIM, unpublished work), and included one recent paper published subsequent to the MEDLINE search (25). One other study, by Liuzzo et al. (26), compared the prognostic role of cTnT against C-reactive protein and serum amyloid A protein but was excluded from the metaanalysis because of unresolved questions concerning the applicability of the cTnT results (27). For the metaanalysis based on the need for revascularization, we used one letter (23), two abstracts (20, 24), and one unpublished manuscript (Stubbs et al.).

In several of these studies, subpopulations were selected for the metaanalysis. The criteria for selection were: (a) patients with ischemic heart disease for which an AMI was ruled out, and (b) populations for which clinical follow-up information was available. In the studies of Hamm et al. (27), Seino et al. (23), Abbas et al. (24), and Stubbs et al. (unpublished), all study patients were diagnosed with UA and were included in our metaanalysis without distinguishing between Braunwald subclassifications of UA. The two reports of Ravkilde et al. (16, 25) included 127 and 124 patients, respectively, with ischemic heart disease but without AMI. The frequency of UA in these two reports was 65% and 58%, respectively. In the report of Burlina et al. (22), follow-up data were reported in 28 of the 32 patients with UA, so these 28 were included in this study. Patients with AMI, nonischemic heart disease, and skeletal muscle injuries were excluded. As re-
ported in the abstract of Benjamin et al. (20), 25% of their non-AMI patients with chest pain had UA.

Statistical Analysis

Clinical sensitivity, specificity, and 95% confidence intervals (CIs) for data from published reports were determined by using standard formulas (28). All other data were entered into a personal computer-based statistical package (Crunch ver. 4.0; Crunch Software, Oakland, CA). χ² (and corresponding P values) and odds (relative risk) ratios were computed with the logistic program available on this package. SROC curves were prepared as described by Littenberg and Mushlin (29). The SROC is plotted from the true-positive (TPR) vs true-negative rates (FPR) obtained from each study. A best-fit curve is computed from the logit transformations of TPR and FPR by use of a weighted linear least-squares regression (29). We used the REGRESSION program available on Crunch to determine the slope (b) and intercept (i) of this regression. The best-fit curve is computed by back-transforming the slope and intercept into the following equation:

\[
TPR = \left[ 1 + \left( e^{i/b} \times (FPR/1 - FPR)^{(i/b)(1-b/i-1)} \right) \right]^{-1}
\]

(1)

Results

Troponin T in Non-AMI

Figure 1 illustrates the clinical sensitivity and specificity for seven individual studies and unweighted cumulative results comparing CK-MB with cTnT for retrospective (12–48 h) diagnosis of AMI. No significant difference was observed when CK-MB was compared with cTnT for clinical sensitivity: 96.8% (CI 95–98%) vs 98.2% (CI 97–99%), respectively. In contrast, the clinical specificity for CK-MB, 89.6% (CI 87–92%), was significantly greater (P <0.001) than that for cTnT, 68.8% (CI 66–72%). Patients with minor myocardial injury will produce positive cTnT results at the cutoff concentrations used in these studies (17).

Figure 2 shows the SROC curves. The weighted linear regression of the plot of d [logit (TPR) - logit (FPR)] vs s [logit (TPR) + logit (FPR)] produced the following equations: d = 0.355s + 4.81 for CK-MB, and d = 0.315s + 5.43 for cTnT. The slope and intercept of these equations were entered into Eq. 1 and the curves were plotted. However, we drew the curves only through the regions where the data were reported and did not extrapolate them to the origin. As shown in Fig. 2, at given clinical sensitivity between 90% and 95%, the clinical specificity for cTnT was slightly better than for CK-MB. However, at the very high sensitivities that were produced with use of the low cTnT cutoff concentrations recommended by the manufacturer (0.1 or 0.2 μg/L), the specificity of cTnT was lower than for CK-MB (the two curves cross each other).

Troponin T in Non-AMI

Figure 3 (top) shows the cumulative metaanalysis (running totals for odds ratios) for the use of cTnT in UA and in non-AMI patients for prediction of AMI or death as each study is chronologically added to the total. Table 1 lists the individual odds ratio for each study. In the first study, by Hamm et al. (21), the data indicate that UA patients with high cTnT had 21.7-fold increased odds of developing AMI than did UA patients with normal cTnT (CI 2.6–180). In the study by Ravnkilde et al. (16), the odds ratio was 4.1 (CI 3.0–17.7; Table 1). Although they used log-rank statistics and found cTnT to be a significant prognostic risk factor (P = 0.025), the odds ratio and χ² statistics used throughout this report showed only marginal significance for cTnT as a prognostic risk factor for AMI (the 95% CI for the odds ratio overlaps with an odds ratio of 1.0, and P =0.05). However, when the data were combined with those from the Hamm et al. study, the cumulative odds ratio was 8.5 (CI 2.7–26.6; P <0.005), demonstrating statistical significance (Fig. 3, top). Adding in the combination of the next three studies brought the cumulative odds ratio up to 10.8 (CI 4.6–25.6). When we added the two most recent studies, however, the individual odds ratios of 5.9 and 2.1 (Table 1) lowered the cumulative odds ratio for the

![Fig. 1. Metaanalysis of CK-MB and cTnT for retrospective diagnosis (12–48 h after chest pain) of AMI.](image)

References listed for CK-MB apply also to cTnT. (A) CK-MB cumulative clinical sensitivity (95% CI): 96.8% (95–98%), specificity 89.6% (87–92%). (B) cTnT cumulative sensitivity 98.2% (97–99%), specificity 68.8% (66–72%).

![Fig. 2. SROC curves for the retrospective diagnosis of AMI.](image)

Each symbol represents one single report for CK-MB (■) and cTnT (○).
Table 1. Individual and cumulative odds ratios of cTnT for development of AMI or death.

<table>
<thead>
<tr>
<th>Study</th>
<th>Follow-up interval</th>
<th>AMI/total</th>
<th>cTnT Odds ratio</th>
<th>cTnT Odds ratio (95% CI)</th>
<th>x²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamm et al. (21)</td>
<td>Hosp period</td>
<td>High</td>
<td>10/33</td>
<td>21.7 (2.6–180.5)</td>
<td>8.15</td>
<td>0.0043</td>
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<td>Ravkilde et al.</td>
<td>6 months</td>
<td>Low</td>
<td>1/51</td>
<td>4.1 (1.0–17.7)</td>
<td>3.84</td>
<td>0.0502</td>
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<tr>
<td>Seino et al. (23)</td>
<td>Hosp period</td>
<td>Medium</td>
<td>2/14</td>
<td>1.0</td>
<td>c</td>
<td></td>
</tr>
<tr>
<td>Burlina et al.</td>
<td>Hosp period</td>
<td>Low</td>
<td>5/16</td>
<td>1.0</td>
<td>c</td>
<td></td>
</tr>
<tr>
<td>Abbas et al. (24)</td>
<td>3 weeks</td>
<td>High</td>
<td>8/27</td>
<td>14.2 (3.4–58.3)</td>
<td>13.50</td>
<td>0.0002</td>
</tr>
<tr>
<td>Ravkilde et al.</td>
<td>28 months</td>
<td>Medium</td>
<td>8/25</td>
<td>5.9 (1.6–21.48)</td>
<td>7.38</td>
<td>0.0066</td>
</tr>
<tr>
<td>Stubbs et al.</td>
<td>34 months</td>
<td>Low</td>
<td>22/82</td>
<td>2.1 (1.1–4.2)</td>
<td>4.62</td>
<td>0.0315</td>
</tr>
<tr>
<td>Cumulative</td>
<td></td>
<td>High</td>
<td>59/221</td>
<td>4.3 (2.8–6.8)</td>
<td>41.12</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

* Mean or median follow-up duration. "Hosp period" indicates that the follow-up period was while the patient was hospitalized.

** Presence of AMI or death/total number of subjects studied in this group.

*** Could not calculate due to absence of AMI or death in the low cTnT group.

that patients with a transient rise in total CK had a higher 1-year incidence of death and recurrent nonfatal AMI than those without such increases [odds ratio 4.3 (CI 1.5–12.2)]. Katus et al. (32) used cardiac myosin light chains and documented death or AMI in 5 of 22 patients with an abnormal value, whereas none of 20 patients with a normal value for myosin light chains had these complications. For use of CK-MB (mass assay) in studies on risk stratification, results have been equivocal. Using the rate of change for serial samples of CK-MB, Pettersson et al. (33) and Ravkilde et al. (34) reported data that produced odds ratios of 67.8 (CI 10–458; 53 patients) and 9.7 (CI 1.86–51.0; 65 patients), respectively. Using a sensitive mass assay for CK-MB (NovoClone) and a low cutoff value (6 μL/L), Ravkilde et al. (25) also found good correlation between CK-MB concentration and outcome after 28 months [odds ratio 8.5 (CI 2.1–34.3)]. In contrast, they found no significant correlation when the cutoff concentration was 8 μL/L [odds ratio 2.7 (CI 0.71–9.9)], consistent with Markenvard et al. (35), who used a cutoff concentration of 10 μL/L for the NovoClone assay and reported data that produced an odds ratio of 0.54 (CI 0.14–2.1). In another study, no correlation [ratio 1.00 (CI 0.08–13.2)] was also found for 131 patients by an immunoassay for CK-MB (Opus Plus) at cutoff concentrations ranging from 5 to 10 μL/L (Wu AHB, Abbas SA, Green S, Pearsall L, Dhakam S, Onoroski M, et al., unpublished work).

The use of low cutoff concentrations for cardiac markers will increase the clinical sensitivity of the assays for detection of minor myocardial injury. However, for CK-MB and other markers such as myosin light chains and myoglobin, which are not entirely specific for cardiac injury, there is a limit as to how low the decision limit can be decreased without significant degradation of clinical specificity. Blood from normal individuals exhibit some baseline concentrations for these markers, which are released during normal skeletal muscle turnover; therefore, use of low cutoff limits will produce false-positive results.

cTnT (and presumably troponin I) may be a more useful cardiac marker for risk stratification of UA and other non-AMI patients because assays of cTnT are more specific towards cardiac injury than are any of the aforementioned markers (36, 37). A low cutoff concentration can be used to differentiate between patients with no injury (low risk) from those with minor injury (high risk) (17). The current database of studies for predicting AMI or death, however, is still very limited—with only seven reported studies dealing with only 699 total patients. Nevertheless, the cumulative metaanalysis shows that patients with high cTnT have a fourfold higher odds for development of AMI or death. If the data on long-term follow-up are excluded from the metaanalysis, the positive cTnT odds ratio for a poor outcome during the first 6 months after cTnT testing is roughly 11-fold that of a patient with a negative serum cTnT value (Fig. 3, top). At longer follow-up periods, the risks between troponin-positive and troponin-negative groups decrease as the results of the cTnT-positive and cTnT-negative patient groups begin to normalize. All patients with ischemic heart diseases have the potential of developing complications, given enough time. Administration of aggressive therapies should be prioritized to those identified as having immediate high risk (within 6 months) for a poor outcome.

The metaanalysis of cTnT concentrations to predict the need for cardiac revascularization is fairly consistent. Patients with high cTnT were generally more seriously ill than those with a normal cTnT. However, the decision to perform surgery depends on both the availability of facilities and the subjective opinions of attending cardiologists. Some patients with high cTnT may be too ill or have too much irreversible damage to warrant the risks from these procedures. As with any laboratory test, management decisions must be based on an assessment of all pertinent clinical and laboratory information available. Considering that the data used to construct this metaanalysis were from clinical practices that likely differ greatly from another (study sites in Japan, the UK, and the US), the cumulative odds ratios shown in Fig. 3 (bottom) do not dramatically change.

The acceptance of cardiologists for risk assessment by cTnT will probably require large prospective clinical studies. Recently, the Department of Health and Human Services published clinical practice guidelines for diagnosing and managing UA (38). Table 8 of those guidelines, which lists factors important in the classification of short-term risk for death or nonfatal AMI, does not include laboratory tests of irreversible damage.

<table>
<thead>
<tr>
<th>Study</th>
<th>Follow-up intervala</th>
<th>Revasc./totalb</th>
<th>Odds ratio (95% CI)</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benjamin et al. (20)</td>
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<td>High cTnT</td>
<td>10/45</td>
<td>40</td>
<td>1.8–8.5</td>
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<td>Seino et al. (23)</td>
<td>Hosp period</td>
<td>Low cTnT</td>
<td>42/622</td>
<td>3.0</td>
<td>1.2–7.1</td>
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<tr>
<td>Abbas et al. (24)</td>
<td>3 weeks</td>
<td></td>
<td>38/104</td>
<td>2.0</td>
<td>1.0–4.0</td>
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<tr>
<td>Stubbs et al.d</td>
<td>34 months</td>
<td></td>
<td>26/121</td>
<td>4.4</td>
<td>3.0–6.5</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
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<th>χ²</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Cumulative</td>
<td></td>
<td></td>
<td>106/555</td>
<td>4.4</td>
<td>3.0–6.5</td>
</tr>
</tbody>
</table>

* Mean or median follow-up duration. "Hosp period" indicates that follow-up period was the while the patient was hospitalized.

a Number of patients undergoing coronary artery revascularization (angioplasty or bypass surgery)/total number of patients in this group.

c Could not calculate due to absence of revascularization in the low-cTnT group.


Table 2. Individual and cumulative odds ratios of cTnT for predicting need for cardiac revascularization.
in this risk assessment. We suggest that considerations be given to inclusion of cTnT as a prognostic risk factor. Whether or not cardiac troponin I will exhibit similar prognostic capabilities remains to be determined.

Properly constructed metaanalyses permit more objective interpretation of data than do narrative review articles. The analyses are still biased, however, given that the criteria for including or excluding a published report are somewhat subjective. Nevertheless, if inconclusive results are obtained after a metaanalysis, suggesting the need for further studies, the objectives and review of data within the metaanalysis may still provide a template for further clinical trials.

References