Sequential Assessment of Troponin in the Diagnosis of Myocardial Infarction

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ABSTRACT

According to the American Heart Association, cardiovascular disease accounts for more than one third of all deaths in the United States. [1] The purpose of this retrospective case-control study was to determine which sample taken in a sequential draw was most important in diagnosing an acute myocardial infarction (AMI). One-hundred subjects were selected from a convenience sample. The "risk" of AMI diagnosis was modeled using binary multiple logistic regression. Overall, 78% (39 out of 50 cases) were diagnosed with an AMI at $T_{initial}$. Clearly, the initial cTnI assay is the most critical of the four sequential time points for the accurate assessment of the presence or absence of an AMI. Most importantly, sequential troponin testing increased the ability to diagnose AMI by 10-fold.

ABBREVIATIONS: ECG - electrocardio-gram, CK - creatine kinase, AMI – acute myocardial infarction, cTnI - cardiac troponin I, CSRA - Central Savannah River Area, STEMI - ST-elevation myocardial infarction

INDEX TERMS: Acute myocardial infarction, Troponin I, Electrocardiogram

Clin Lab Sci 2013;26(2):95

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The American Heart Association (2008) reported that cardiovascular disease accounted for more than one third of all deaths in the United States.[1] An early and accurate diagnosis of myocardial infarction (MI) has continued to be a safety issue for patients who present to the emergency department with chest pain. Previously, such patients were evaluated solely based on their past history, physical examination, electrocardiogram (ECG), and assessment of creatine kinase (CK) and CK-MB fractions.[2] As advancements in research continue to evolve, many hospitals have adopted the assessment for the cardiac protein troponin I in sequential blood sampling as a more rapid biomarker to correctly diagnose an AMI. The purpose of our study was to determine which sample out of multiple samples in a sequential draw was most predictive in the differential diagnosis of acute myocardial infarction for patients in an emergency department of the Central Savannah River area.

Polanczyk et al (1998) conducted a cohort study to

evaluate the diagnostic and prognostic value of cardiac troponin I in chest pain patients over age 30 (mean age of 61) who presented to the emergency department of the Brigham and Women's Hospital between July 1994 and June 1995.[2] Blood sampling occurred after admission and every 8 hours for at least a 24 hour period to examine cardiac enzyme measurements for CK and CK-MB according to a standard; and likewise, cardiac troponin I (cTnI) was measured to rule out myocardial infarction (MI). The investigators analyzed all samples on the Stratus instrument with a lower level of detection (LLD) for cTnI at 0.4 ng/mL and an upper reference limit (URL) of cTnI at 1.5 ng/mL. Although determining cTnI was not the standard diagnostic tool for this hospital, the researchers concluded that it showed better performance than CK-MB mass assay for ruling out MI.

Kontos et al (2000) examined troponin I (cTnI) in relation to cardiac events in a large, heterogeneous, nonselected patient group for exclusion of myocardial infarction (MI) at the Medical College of Virginia Hospital's Emergency Department.[3] They collected samples at the time of admission, at 8 hours, and continued at 6 to 8 hour intervals for patients who had recurrent symptoms indicative of MI. cTnI was measured in plasma on the Opus Magnum Analyzer using 0.5 ng/mL as the lower limit for detectability and 2.0 ng/mL as the manufacturers' suggested diagnostic value for MI. The predictive value demonstrated that when cTnI was used as part of an 8hr rapid diagnostic protocol, it had a high sensitivity (92 to 98%) for identifying patients who had an AMI.

The purpose of the study by Straface et al (2008) was to develop a more rapid and thorough screening protocol in the ED with multimarkers for MI to eliminate false positive results and unacceptable false negative results.[1] The authors compared a rapid, point-of-care multimarker protocol with a single serial troponin I (cTnI) draw only. The conclusion of this study was that the new rapid multimarker protocol seemed to be superior to just the serial troponin draw alone approach for managing patients who present to the ED with chest pain or AMI.

From January 2007 through December 2008, Keller et al (2009) evaluated the diagnostic accuracy and clinical usefulness of a sensitive troponin I assay in a multicenter study for early diagnosis of MI in 1818 consecutive chest pain patients who presented to three German study centers.[4] The sensitive cTnI assay provided an overall 90.7% sensitivity and 90.2% specificity regardless of the time that had elapsed between chest pain onset and hospital admission. The researchers concluded that the elevated cTnI values measured at the time of admission with the sensitive assay provided diagnostic accuracy in early discrimination of MI.

Madsen et al (2006) performed a study to define the time course of cardiac troponin (cTnI) degradation in patients with acute ST-elevation myocardial infarction (STEMI).[5] Using the ASSENT-2 study, the researchers randomized 26 males, ages 33-72, hospitalized with STEMI to 2 different thrombolytic drugs (tenecteplase and alteplase) within 6 hours after onset of their symptoms.[6] Blood samples were drawn just before initiation of thrombolysis and at 30 minutes intervals (7 samples per patient). cTnI analysis was done by Western blot. The results were that all patients exceeded the cTnI cutoff for MI at admission. The study concluded that cTnI was detectable approximately 90 minutes after the onset of symptoms.

The purpose of this study was to determine which sequential cardiac troponin I (cTnI) sample was most predictive for accurately diagnosing an MI. The investigators expected that patients experiencing chest pain were admitted from the emergency department observational unit (EDOU) after their cTnI concentrations were above a normal reference range, and patients were not withheld for further cardiac observation if the cTnI assessment remained within normal limits after sequential testing. The covariants for this study were cardiovascular risk factors such as race, age, and gender, along with the protocol. The independent variables for the study were the times to the MI event (i.e. $T_{initial}$, T_{3hr}, T_{6hr}, and $T_{8\text{-}12hr}$). The dependent variable for the study was the presence or absence of an MI event. The investigators correlated the data statistically with the findings using a logistic regression model. Data was characterized by event-times which were determined from a specific initial time that reflected a starting point for cTnI assessment until the time that a patient was diagnosed with an MI. This data was limited to a 12-hour period wherein the investigators were concerned with any point in time

that an individual had a risk, or hazard, of having an MI. The binary multiple logistic regression model is a standard statistical model of analysis that named the serial blood sampling protocols as a dichotomous variable with two levels: Protocol Not Followed and Protocol Followed to aid the investigators in understanding the significant time points when an MI is diagnosed. The χ^2 and two-sample t-tests were conducted to determine case and control group differences in the proportions and means for the covariants. The investigators ascertained which sample was critical in the series of draws for cTnI as well as the significance of age and whether the protocol was followed or not in the determination of MI.

METHODS

The study was a retrospective case-control study of 50 consecutive patients who presented to the emergency department of Georgia Health Sciences Health Systems with angina pectoris, commonly known as chest pain, and underwent sequential blood sampling for cardiac troponin I (cTnI) analysis in an effort to diagnose acute myocardial infarction (AMI). The subjects were residents of Richmond and Columbia counties in Georgia and those who resided in the surrounding Central Savannah River Area (CSRA). Potential cases were men and women who were 40 – 79 years of age and were diagnosed with an MI between January 1, 2010 and December 31, 2010. Control subjects were of the same geographic regions as the cases and similar for race, gender, and age criteria. The MI cases and controls were identified and selected from the Georgia Health Sciences Health Systems' medical records database using Powerchart from a patient work list based on specific ICD-9 code 786.50 for chest pain NOS (controls) and ICD-9 code 410.71 for AMI (cases). Medical records were reviewed to retrieve demographic characteristics and clinical history of the study population, cardiovascular risk factors, and laboratory analyses such as cTnI concentrations, admission status, MI diagnosis, and discharge. Subjects were eligible to be a case if the medical record showed a final diagnosis of MI based upon laboratory findings of the initial, second, third, or fourth cTnI assessment. Subjects who presented in the ED with a final diagnosis of chest pain were eligible to be controls. cTnI was measured either directly by using a point-of-care device (I-Stat, Abbott Laboratories) or a traditional method (Centaur, Siemens Health Systems) of testing in the core laboratory.

All data was analyzed statistically by a binary multiple logistic regression model. The binary multiple logistic regression model tested the covariable effects of each factor (e.g. race, gender, and age) and gave an odds ratio. An odds ratio was also calculated for the protocol being followed or not followed. Odds ratios represented the increased or decreased risk of an MI event at the points of cTnI assessment. The risks for the subgroups were assumed to be proportional in the odds ratios. Therefore, values above one (>1.0) indicated a higher risk, values below one (<1.0) indicated a lower risk, and values equal to one (=1.0) indicated that there was no increased or decreased risk of having an MI. Both race and gender covariables were selected equally for cases and controls. That is, equal proportions of males and females and equal proportions of Caucasians and African Americans were randomly chosen for both case and control subjects. According to the AMI diagnostic protocol for sequential cTnI testing, blood draws were to be taken when patients initially presented to the emergency department (ED) with angina pectoris (chest pain) and at 3 hours, 6 hours, and from 8 to 12 hours after their initial cTnI assessment. A dichotomous predictor variable of the chest pain pathway protocol was created with two levels: protocol not followed and protocol followed. All statistical tests were conducted at the $\alpha = 0.05$ significance level. The objective was to identify the strength time interval that was most predictive and diagnostic of an MI based on the sequential assessment of cTnI.

RESULTS

Table 1 shows the characteristics of cases with a diagnosis of acute myocardial infarction (AMI) at the time of discharge. Table 1 also displays the characteristics of the controls who had a discharge diagnosis of chest pain. Control patients also received the cTnI testing protocol. As stated previously, there were equal proportions of males and females and equal proportions of Caucasians and African Americans who were chosen for both case and control subjects. Table 2 displays the odds ratios in the column (Exp (B)) and the 95% confidence intervals representing upper and lower limits for the dichotomous predictor variable (protocol followed) and the covariable (age). Age is a significant contributor to risk of having an AMI in that patients diagnosed were generally older. Following the protocol is a significant factor in being able to rule-in an AMI with the ability to detect an AMI having a 10-fold

increase when the protocol was followed. The Hosmer and Lemeshow goodness-of-fit test was conducted on the multiple logistic regression data to determine what percentage of cases and controls were correctly classified.[7] Table 3 is a 2x2 contingency table representing either correct or incorrect classifications of cases and controls using the variables age, gender, race, and protocol. Seventy-seven percent of the cases and controls were correctly classified. The test indicated a good statistical fit of the research model (χ^2 = 10.6, 8 df, p = 0.226).

Table 1. Characteristics of study subjects with and without a diagnosis of acute myocardial infarction (AMI) at discharge

Variables	No AMI (controls) n = 50	AMI (cases) n = 50	P-value*
Age (yrs)	54.0 ± 8.414	61.5 ± 7.691	< 0.001
Gender			
Female	25 (50)	25 (50)	1
Male	25 (50)	25 (50)	
Race			
White	25 (50)	25 (50)	1
Black	25 (50)	25 (50)	
Protocol			< 0.001
Not Followed	34 (68)	11 (22)	
Followed	16 (32)	39 (78)	

Note: Table entries for the quantitative variables are of the form mean ± standard deviation. Table entries for the qualitative variables are of the form count (percentage).
* Determined by t-test for quantitative variables and χ^2 test for qualitative variables

Table 2. Final statistics of the multiple logistic regression analysis of the presence of acute myocardial infarction (AMI) diagnosed at discharge

Variable	B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I. for Exp (B) Lower	Upper
Age	.137	.034	15.974	1	.000	1.146	1.072	1.226
Protocol (Not Followed)	2.342	.544	18.503	1	.000	10.402	3.578	30.237
Constant	-9.586	2.220	18.645	1	.000	.000		

Note: Reference categories for the dichotomous qualitative variables are in parentheses.

Three-hour cTnI assessments were not done for 23 (46%) controls and were not done for 48 (96%) cases. Six-hour cTnI assessments were not done for 16 (32%) controls and were not done for 26 (52%) cases. Eight-to-12-hour cTnI assessments were not done for 16 (32%) controls and were not done for 17 (34%) cases. There were only 11 out of 50 cases with an initial cTnI level < 0.5 ng/mL who were found on subsequent testing to have at least one cTnI level > 0.5ng/mL. Therefore, these 11 subjects were diagnosed as having an AMI.

Table 3. Classification table employing results of the multiple logistic regression prediction table with a cut value of 0.5

Observed		Predicted: Acute Myocardial Infarction Control	Case	Percent Correct
Acute Myocardial Infarction	Control	38	12	76
	Case	11	39	78
Overall Percentage				77

Seventy-eight percent (39 out of 50 cases) were diagnosed with an AMI at $T_{initial}$. None of these cases had a T_3 performed which was a deviation from the protocol. Of the remaining 11 cases, 7 (14%) were diagnosed with an AMI at T_6. The remaining 4 cases (8%) were diagnosed with an AMI at $T_{8\text{-}12}$ (Table 4).

Table 4. What is the most significant draw in the series of cTnI analyses for the diagnosis of AMI?

n = 50	$T_{initial}$	T_3	T_6	$T_{8\text{-}12}$
number diagnosed	39	0	7	4
% diagnosed	78	0	14	8

Note: Table entries show that most AMIs were diagnosed on the initial serial draw.

DISCUSSION

What is the most significant draw in the series of cTnI analyses for the diagnosis of AMI? From the collected data, the investigators conclude that the initial cTnI blood draw is clearly the most important of the four sequential time points for the accurate assessment of the presence or absence of AMI for those presenting to the emergency department with angina pectoris. Table 4 highlights this observation showing that 78% of the cases were diagnosed at the $T_{initial}$.

Table 1 reflects significant group differences in whether or not the sequential cTnI assessment protocol was followed. The protocol was not followed 32% of the time for controls as compared to 77% of the time for cases. Cases that were not followed were missing at least one cTnI assessment between the initial cTnI and when a positive result was obtained. If the AMI was diagnosed

on the initial or any cTnI in sequence, the protocol was considered followed. There was a 10-fold increase in the ability to diagnose an AMI if the protocol was followed compared to not followed (Table 2, Odds ratio 10.4, p-value < 0.001). Also note from Table 2, based on the 95% CI of the odds ratio, there was a maximum of a 30-fold increase of detecting an AMI if the protocol was followed. Therefore, it is critical that the AMI diagnostic protocol for sequential cTnI testing be followed consistently.

The covariable age demonstrated a statistically significant difference between the cases and controls. The risk of an AMI increased by 15% with each year of advancing age (Table 2, Odds ratio = 1.146, p-value < 0.001). Subjects with an AMI diagnosis were, on average, approximately 7.5 years older than control subjects. This shows that older individuals experiencing chest pain are more likely to be having an MI than younger patients. Race and gender may also be a factor; however this experimental model controlled for both.

Using the predictor variable (protocol followed/not followed) and the covariable (age), allowed the investigators to correctly classify cases and controls as having or not having an AMI 77% of the time using the Hosmer and Lemeshow goodness-of-fit test (Table 3). Out of 50 subjects in the control group, 38 were classified as true controls with the remaining 12 control subjects resembling cases. Out of 50 subjects in the case group, 39 were classified as true cases and the remaining 11 subjects in the case group resembled controls (Table 3). The Hosmer and Lemeshow goodness-of-fit test indicates a good fit of the multiple logistic regression model (χ^2 = 10.6, 8 df, p = 0.226).[7] The clinical findings of this study support that the AMI diagnostic protocol for sequential cTnI testing be followed in the future to improve the ability to diagnose an AMI quickly. However, these results also show that four serial draws are not necessary to diagnose an AMI. Georgia Regents University has changed their protocol to include only an initial draw and a T_6.

LIMITATIONS

A limitation of this study is that the protocol was not followed in all cases and controls. In addition, a small cohort of only 50 patients may not be representative of the population. The study excluded other biomarkers and assessments such as CK-MB and myoglobin. Finally, all collected data only reflected up to 12 hours after ED admission such that, AMI occurrences after 12 hours were not noted and patients discharged or readmitted before 12 hours were not followed.

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