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Stability of Cardiac Troponin-I in Whole Blood and Plasma in Patients with Acute Myocardial Infarction

Akut Miyokard Enfarktüslü Hastalarda, Tam Kan ve Plazma Kardiyak Troponin-I Stabilitesi

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ABSTRACT

Introduction: In this study, we aimed to investigate the short-term stability and variability of cardiac troponin T (cTnI) in ethylenediamine tetraacetic acid (EDTA) whole blood, heparinized whole blood, and EDTA plasma.

Methods: Thirteen patients with myocardial infarction were included. Venous blood samples (24 mL) from all patients were collected into vacuum tubes with EDTA and heparin (Becton Dickinson-USA), and analyses were performed on four different groups (one group for repeatability and three groups for stability).

Results: There was no statistically significant difference in cTnI concentrations at baseline, 2, 4, 6, 12, 24, and 48 hours between the heparinized whole blood, EDTA whole blood, and EDTA plasma groups (group 2) ($p>0.05$). In the heparinized whole blood and EDTA plasma groups, there was a statistically significant difference between cTnI concentrations at baseline, 2, 4, 6, 12, 24, and 48 hours. In the EDTA whole blood group, there was no statistically significant difference between any time points.

Conclusion: The interclass and intraclass correlation coefficients of the EDTA whole blood group were sufficiently high, which indicates better stability. EDTA whole blood samples are preferable for cTnI measurement because they are stable for 48 hours. EDTA-containing tubes are easy to find in clinical laboratories and do not need to be centrifuged, which saves time and effort.

Keywords: Cardiac troponin-I, acute myocardial infarction, plasma, whole blood

ÖZ

Amaç: Bu çalışmada EDTA tam kan, heparinize tam kan ve EDTA plazmasındaki kısa-süreli kardiyak troponin T (cTnI) stabilitesini ve değişkenliğini araştırmayı amaçladık.

Yöntemler: Miyokard enfarktüsü geçiren 13 hasta dahil edilmiştir. Tüm hastalardan 24 mL venöz kan örnekleri EDTA ve heparinli vakum tüplerine alındı (Becton Dickinson-ABD) ve analizler 4 farklı grupta (tekrarlanabilirlik için 1 grup ve stabilite için 3 grup) gerçekleştirildi.

Bulgular: Heparinize tam kan, EDTA tam kan ve EDTA plazma grupları arasında başlangıç, 2., 4., 6., 12., 24 ve 48. saat cTnI konsantrasyonları arasında istatistiksel olarak anlamlı bir fark yoktu ($p>0,05$). Heparinize tam kan ve EDTA plazma gruplarında, başlangıç, 2., 4., 6., 12, 24, 48 saat cTnI konsantrasyonları arasında istatistiksel anlamlı fark vardı. EDTA tam kan grubunda tüm saatler arasında istatistiksel olarak anlamlı fark yoktu.

Sonuç: EDTA tam kan grubunun sınıflar arası ve sınıflar arası korelasyon katsayıları yeterince yüksekti, bu da daha iyi bir stabilite olduğunu göstermektedir. EDTA tam kan örnekleri cTnI ölçümünde tercih edilebilir, çünkü 48 saat stabildir. EDTA içeren tüplerin klinik laboratuvarlarda bulunması kolaydır ve santrifüjlemeye gerek kalmaz, zamandan ve emekten tasarruf sağlar.

Anahtar Kelimeler: Kardiyak troponin-I, akut miyokard enfarktüs, plazma, tam kan

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Introduction

Cardiac troponin-I (cTnI) is widely used as the gold standard marker of myocardial cell damage. The quantitative measurement of cTnI is very important for the diagnosis and treatment of myocardial infarction (MI). Following myocardial cell damage, cytosolic troponin is released first, and as further damage occurs, troponin present in the sarcomere is released into the circulation. For this reason, troponin is both an early and late marker of acute myocardial infarction (AMI).

The kinetics of cTnI release after damage are as follows: levels of cTnI start increasing 4-9 hours after AMI and peak at 12-24 hours. They can remain elevated for up to 14 days.

A rise in circulating cTn with at least one value above the 99th percentile upper reference limit is indicative of the presence of AMI and is useful within the particular diagnosis of ST segment elevation but especially non-ST-elevation myocardial infarction (1). Measurement of cTn plays a critical role in the rapid assessment of patients admitted to the emergency department with acute coronary syndrome (2). By using troponin-specific antibodies, cTn levels can be determined in the blood. More than one assay is available for cTnI, and each antibody measures different epitopes and fragments (3). Therefore, there is no standardization between assays. Different assays have different cut-off limits. Also, the complex release of cTn (in the form of cTn-T, I, and C complex) may cause different results between measurements, because some of the antibodies used in the measurements are not able to recognize some of the cTn forms in the complex, which cause lack of standardization in cTnI measurement (4,5). cTnI measurements are influenced by multiple factors, including proteolytic degradation, heparin, heterophile antibodies, human-animal antibodies, autoimmune antibodies, rheumatoid factors, cTnI-specific autoantibodies, hemolysis, and fibrin (6). These variations in troponin measurements can cause diagnostic problems, so the stability of troponin is crucial, especially in the area of AMI.

In this study, the aim was to evaluate how the cTnI test results of patients diagnosed with MI were affected when blood samples were collected into tubes containing different anticoagulants and when analyzed under different storage conditions.

Methods

A total of 13 patients 18 years and older and diagnosed with AMI in the emergency room were included in the study prospectively. MI is diagnosed when blood levels of sensitive and specific biomarkers such as cTn or CK-MB are increased in the clinical setting of acute myocardial ischemia. Patients with chronic kidney disease, acute infection, congestive heart failure, or chronic obstructive pulmonary disease at admission were excluded because of the risk of false positivity. This study was approved by the University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital Local Ethics Committee (approval number: 2014/09/01, date: 14.07.2014). All participants' rights were protected, and written informed consent was obtained before the procedures, according to the Helsinki Declaration (2013). Venous blood samples (24 mL) were collected from all patients into vacuum tubes with ethylenediamine tetraacetic acid (EDTA) and heparin (two EDTA tubes BD, 10 mL K2 EDTA, Cat. No: 367525 and two heparin tubes BD, 4 mL lithium heparin, Cat. No: 367883).

The first samples that arrived in the laboratory were defined as baseline cTnI value (0 minutes), and troponin-I analyses were completed within 30 minutes after arrival. The whole blood samples collected into tubes with EDTA and heparin were kept at room temperature, and troponin-I analyses were repeated after 2, 4, 6, 12, 24, and 48 hours. Additional EDTA samples were centrifuged immediately, and the first aliquoted plasma samples were analyzed for troponin-I, whereas the other aliquots were frozen and kept at -20 °C. Troponin-I analyses were repeated after 2, 4, 6, 12, 24, and 48 hours from frozen plasma samples.

cTnI was analyzed with the AQT90 analyzer closed-tube system (Radiometer Medical Aps, Denmark) using the time resolve fluorometry method. The minimum sample volume was 2 mL.

Analyses were performed on four different groups (one group for repeatability and three groups for stability). The first group of blood samples (n=10) were analyzed as whole blood with EDTA, and all repeatability studies were completed within 6 hours on the day of collection. All analyses were performed 10 times for each patient.

The second group of whole blood samples with EDTA (n=10) was centrifuged at 1000 g for 5 minutes within 30-60 minutes, and plasma samples were obtained. Baseline levels were recorded, then other plasmas were melted after the appropriate incubation period, and all analyses were completed within 30 minutes.

The third group of blood samples with EDTA (n=7) and the fourth group with heparin (n=10) were analyzed, and after measurement of baseline levels, the next set of samples was analyzed within 30 minutes after the appropriate incubation period.

Statistical Analysis

In this study, statistical analyses were performed with the Number Cruncher Statistical System 2007 Statistical Software (Utah, USA) package program. In the data analysis, descriptive statistical methods (mean, standard deviation) were used, as well as repetitive variance analysis in multiple groups, Newman-Keuls multiple comparison test in subgroup comparisons, and One-Way analysis of variance in between-group comparisons.

Intraclass correlation coefficients (ICCs) were calculated for measurement of reliability. The results were evaluated at a significance level of $p<0.05$.

Results

The cTnI levels of a total of 13 AMI patients were tested on three different samples in two different collection tubes at seven different time points. For the reliability of EDTA whole blood measurement (group 1), the results are listed in Table 1. The ICC was calculated, and the reliability coefficient was found to be 0.997 (0.995-0.999). The variability of the measurements was found to be 1.7%, which indicates high reliability of EDTA whole blood measurements (Table 2).

There was no statistically significant difference between cTnI concentrations at baseline, 2, 4, 6, 12, 24, and 48 hours in the EDTA plasma group (group 2), EDTA whole blood (group 3), and heparinized whole blood (group 4) ($p>0.05$) (Table 3). In the EDTA plasma group (group 2), there was a statistically significant change in the cTnI concentration

between baseline and 2, 4, 6, 12, 24, and 48 hours (p=0.028) (Table 3). In the EDTA whole blood group (group 3), no significant difference was observed between cTnI concentrations at baseline and 2, 4, 6, 12, 24, and 48 hours (p=0.107) (Table 3). In the heparinized whole blood group (group 4), there was a statistically significant difference in cTnI concentrations between baseline and 2, 4, 6, 12, 24, and 48 hours (p=0.002) (Table 3).

In multiple comparison analyses of groups 2 and 4, the baseline values were statistically significantly higher than the 12-, 24-, and 48-hour measurements (p=0.048, p=0.049, p=0.013) (Table 4). The 48-hour values were statistically significantly lower than those at 4, 6, 12, and 24 hours (p=0.018, p=0.011, p=0.049, p=0.027), and no significant difference was observed between the other time points (p>0.05) (Table 4). The 4-hour values were significantly lower than the baseline and 12-hour values (p=0.012, p=0.026). The 24-hour values were significantly lower than the 12-hour values (p=0.017). The other time points did not differ statistically significantly (p>0.05) (Table 4).

Discussion

In our study, no significant intraclass and interclass difference in cTnI values was found in EDTA whole blood. The interclass and ICCs of the EDTA whole blood group were sufficiently higher, which indicates better stability compared with the other two groups.

There was also no statistically significant difference between cTnI levels in whole heparinized blood, EDTA whole blood, and EDTA plasma samples at the same hours, which showed a similar safety profile.

No differences in stability were reported between whole EDTA blood, EDTA plasma, and serum (7) or between heparinized whole blood and plasma (8).

In a study by Chapelle et al. (6), heparinized whole blood and plasma samples were collected from 85 patients with suspected MI, and no statistically significant difference was found between the two sample types, as in our study. There were significant differences between intraclass (baseline, 4-, 6-, 12-, 24-, and 48-hour) measurements of the heparin whole blood group (6). Binding of heparin to cTnI may cause

Table 1. Results of patients in EDTA whole blood (group 1)

Sample ID	Number of repeats	Minimum value	Maximum value	Mean ± SD
1	10	0.84	1.1	0.946±0.075
2	10	1.3	1.5	1.41±0.056
3	10	2.8	3.1	2.97±0.094
4	10	3.7	4.1	3.9±0.019
5	10	1.9	2	1.96±0.032
6	10	1.1	1.3	1.18±0.030
7	10	0.53	0.67	0.579±0.042
8	10	1.4	1.5	1.45±0.052
9	8	2.1	2.3	2.175±0.103
10	8	1.3	1.6	1.4±0.092

SD: Standard deviation, EDTA: Ethylenediaminetetraacetic tetraacetic acid

Table 2. Variability of group 1 (EDTA whole blood)

	Variability %	ICC	95% CI	
			Lower bound	Upper bound
EDTA whole blood	1.7	0.997	0.995	0.999

ICC: Intraclass correlation coefficient, CI: confidence interval, EDTA: Ethylenediaminetetraacetic tetraacetic acid

Table 3. Comparison of groups 2, 3, and 4

	Heparinized whole blood (group 4)	EDTA whole blood (group 3)	EDTA plasma (group 2)	p
Baseline	3.25±2.58	2.06±0.91	2.87±2.13	0.753
2. hours	3.11±2.43	2.03±0.93	2.76±1.99	0.866
4. hours	3.06±2.3	2±0.99	2.67±1.95	0.767
6. hours	3.11±2.46	2.02±0.91	2.79±2.06	0.781
12. hours	3.01±2.34	1.92±0.81	2.8±1.96	0.674
24. hours	3.02±2.48	1.88±0.83	2.66±1.84	0.761
48. hours	2.86±2.27	1.99±0.82	2.73±2.03	0.893
p	0.002	0.107	0.028	

EDTA: Ethylenediaminetetraacetic tetraacetic acid

Table 4. Comparison of groups 2 and 4

Multiple comparison test	Heparinized whole blood (group 4)	EDTA plasma (group 2)
Baseline/2. hours	0.173	0.107
Baseline/4. hours	0.107	0.012
Baseline/6. hours	0.169	0.062
Baseline/12. hours	0.048	0.362
Baseline/24. hours	0.049	0.121
Baseline/48. hours	0.013	0.135
2. hours/4. hours	0.429	0.061
2. hours/6. hours	0.931	0.490
2. hours/12. hours	0.084	0.478
2. hours/24.hours	0.480	0.205
2. hours/48. hours	0.025	0.667
4. hours/6. hours	0.719	0.122
4. hours/12. hours	0.238	0.026
4. hours/24. hours	0.310	0.858
4. hours/48. hours	0.018	0.257
6. hours/12. hours	0.066	0.734
6. hours/24. hours	0.141	0.156
6. hours/48. hours	0.011	0.291
12. hours/24. hours	0.611	0.017
12. hours/48. hours	0.049	0.230
24. hours/48. hours	0.027	0.588

EDTA: Ethylenediaminetetraacetic tetraacetic acid

lower measured values by masking specific epitopes. In addition, therapeutic heparin treatment during the early hours of AMI may also interfere with these samples. These effects may give us an idea to explain the intraclass differences in the heparin whole blood group. Also, in the EDTA plasma group, there was a statistically significant intraclass difference.

EDTA, as an anti-coagulant, may cause discrepancies, especially in assays utilizing antibodies that differentially recognize free and complexed cTnI. EDTA can cause partial unfolding of the calcium-dependent troponin complex and changes the three-dimensional structure of the troponin complex by binding calcium. Partial unfolding of the cTnI-TnC complex in the absence of calcium would thus facilitate blockage of some cTnI epitopes by the interfering factor. In the presence of calcium, the cTnI-TnC complex will stay more tightly together, reducing the interaction of the interfering factor with cTnI (9).

No significant difference was observed in cTnI concentrations in the EDTA whole blood group, between baseline and 2, 4, 6, 12, 24, and 48 hours. When compared with the EDTA whole blood group, the heparin whole blood and EDTA plasma groups did not differ significantly. This was the key part of our study. The interclass and ICCs of the EDTA whole blood group were sufficiently high, which indicate better stability.

There is substantial heterogeneity in cTn assays, and different cut-off values are used from one assay to the other and from one laboratory to another. In addition, there are preanalytical and analytic problems associated with samples for the measurement of cTn (10).

All studies must report the specific metrics utilized for the evaluation of cTn in all biomarker papers, compliant with the Standards for Reporting Diagnostic Accuracy guidelines (11).

Conclusion

EDTA whole blood samples are preferred for cTnI measurement, because they are stable for 48 hours. EDTA-containing tubes are easy to find in clinical laboratories, and there is no need for centrifugation, which saves time and effort.

Ethics

Ethics Committee Approval: This study was approved by the University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital Local Ethics Committee (approval number: 2014/09/01, date: 14.07.2014).

Informed Consent: All participants' rights were protected, and written informed consent was obtained before the procedures, according to the Helsinki Declaration (2013).

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