Effect of Hemolysis on Beckman Coulter High Sensitive Cardiac Troponin I Measurement

Beckman Coulter Yüksek Duyarlıklı Kardiyak Troponin I Ölçümü Üzerine Hemolizin Etkisi

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ABSTRACT

Aim: Measurement of cardiac troponins is of great importance in the diagnosis and follow-up of acute myocardial infarction. The diagnostic specificity of myocardial infarction has increased with algorithms including serial troponin measurements. It is important that the measurements in the troponin cut-off values used to evaluate the risk of myocardial infarction in algorithms are reliable. In this study, it was aimed to evaluate the effect of hemolysis on clinical decision concentrations of the high sensitive cardiac troponin I (hs cTnI) test.

Material and Methods: Two pools were created from serum samples with the 99th percentile upper reference value (17.5 ng/L) of hs cTnI and the high-risk cut-off value (50 ng/L) as specified in the European Society of Cardiology 2020 manual. Hemolysate was spiked in both pools with a hemolysis index of 100 to 1000.

The hs cTnI levels in hemolysate-added sera were measured in Beckman Coulter UniCel Dxl 800 immunoassay device. Levels of hemolysis were calculated as percentage change.

Results: Due to hemolysis, hs cTnI values decreased in varying proportions from 3.8% to 20.4% at 99th percentile concentration; values decreased from 4.3% to 20.0% in high-risk cut-off concentration.

Conclusions: In hemolyzed samples, hs cTnI values decrease, this decrease is not considered clinically significant until high hemolysis values. However, it should be kept in mind that a decrease that may occur even in a low hemolysis may lead to erroneous evaluations in clinical decision concentrations.

Keywords: Hemolysis; interference; preanalytical error; troponin

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ÖZET

Amaç: Kardiyak troponinlerin ölçümü akut miyokard enfarktüsünün tanı ve takibinde büyük önem taşımaktadırlar. Seri troponin ölçümlerinin yer aldığı algoritmalar ile miyokard enfarktüs tanısının spesifisitesi artmıştır. Algoritmalarda, miyokard enfarktüs riskini değerlendirmede yer alan troponin cut-off değerlerindeki ölçümlerin güvenilir olması önemlidir. Bu çalışmada, yüksek duyarlıklı kardiyak troponin I (hs cTnI) testinin klinik karar konsantrasyonları üzerine hemolizin etkisini değerlendirmek amaçlandı.

Gereç ve Yöntem: Serum örneklerinden, hs cTnI'nin 99. persentil üst referans değeri (17.5 ng/L) ve Avrupa Kardiyoloji Derneği 2020 kılavuzunda belirtilen yüksek-risk cut-off değeri (50 ng/L) olacak şekilde iki havuz oluşturuldu. Her iki havuza hemoliz indeksi 100'den 1000'e kadar olacak şekilde hemolizat eklendi. Hemolizat eklenmiş serumlarda hs cTnI düzeyleri Beckman Coulter UniCel Dxl 800 immünoassay cihazında ölçüldü. Hemolizden etkilenme düzeyleri yüzde değişim olarak hesaplandı.

Bulgular: Hemoliz nedeniyle hs cTnI değerleri, 99. persentil konsantrasyonunda %3.8'den %20.4'e; yüksek-risk cut-off konsantrasyonunda %4.3'ten %20'ye kadar değişen oranlarda azaldı.

Sonuç: Hemolize örneklerde hs cTnI değerleri azalır, bu azalma yüksek hemoliz değerlerine kadar klinik olarak anlamlı kabul edilmemektedir. Ancak düşük bir hemolizde bile oluşabilecek bir azalmanın klinik karar konsantrasyonlarında hatalı değerlendirmelere yol açabileceği unutulmamalıdır.

Anahtar Kelimeler: Hemoliz; interferans; preanalitik hata; troponin

INTRODUCTION

Acute myocardial infarction (AMI) is an important cardiac emergency that can cause mortality and morbidity (1). Patients with symptoms suggestive of AMI constitute approximately 10% of all patients admitted to the emergency department (2). In addition to clinical evaluation and electrocardiogram (ECG), biochemical tests and imaging methods are used in the diagnosis and monitoring of patients (3, 4). The most sensitive and specific of these biochemical tests are cardiac troponin I (cTnI) and cardiac troponin T (cTnT) (5). In recent years, high-sensitivity cardiac troponins (hs cTn), whose elevation can be detected within 1-2 hours after the onset of AMI symptoms, have been developed compared to conventional troponins (6). Detection of an increase and / or decrease in at least 1 value of cTn above the 99th percentile, with clinical evidence of ischemia, such as chest pain or new ischemic electrocardiogram (ECG) changes, are among the AMI criteria (4). Since the increase in cTn can be seen in other clinical situations, serial troponin measurements are made to increase the specificity of the diagnosis of AMI. Various rapid rule-in and rule-out algorithms have been developed from the measured cTn in serial troponin measurements and the changes calculated between them (delta). The use of algorithms and hs cTn has led to a reduction in diagnosis delays, resulting in shorter stay times in the emergency room and lower costs. One of these algorithms is the 0-1 h algorithm of the European Society of Cardiology (ESC) 2020 guideline (5). Especially in the diagnosis of myocardial infarction without ST segment elevation (NSTEMI) on ECG, hs cTn's are measured at admission and 1 hour after the admission. Risk determination for MI is made according to certain cut-off and delta values of measured troponins.

Hemolysis, which is a major problem of samples especially originating from emergency services, is the most common cause of preanalytical error (7, 8). Rejection of such samples by laboratories will result in retaking samples, delaying results and increasing the workload of employees. It has been reported in various studies that hemolysis causes interference in measurements of both conventional and hs cTn tests (9-15).

Our aim in this study was to evaluate the effect of hemolysis on the 99th percentile value of Beckman Coulter Access hs TnI and the cut-off value for "Rule-in" in AMI, which is included in the ESC 2020 guideline.

MATERIALS AND METHODS

Hemolysis Index Measurement

1 Hemolysis (H) index unit is about 1 mg/dL hemoglobin (Hb). H index was measured in AU 5800 analyzer (Beckman Coulter Inc. Brea, CA, USA).

hs cTnI measurement

Serum hs cTnI was analyzed with Access hs TnI reagent in UniCel DXI 800 (Beckman Coulter Inc. Brea, CA, USA) device by paramagnetic particle chemiluminescent immunoassay method.

As specified in Access hs TnI reagent, limit of detection (LOD) was 2.3 ng/L, the 10% CV limit of quantitation (LOQ) was 5.6 ng/L, and the 99th percentile value was 17.5 ng/L.

Preparation of the hemolysate

Hemolysate was prepared according to the method developed by Meites S.(16). The cells in the whole blood sample taken into the Li-heparin tube were washed 3 times with saline, after adding an equal amount of distilled water, frozen at -80 °C overnight. After dissolving at room temperature, it was centrifuged at 1500 x g for 10 minutes and the supernatant (hemolysate) was separated. The Hb concentration of hemolysate was measured as 19 g/dL with an automatic blood cell counter (1000-XN, Sysmex, Japan).

Forming 99th percentile and high-risk cut-off hs cTnI groups and Interference studies

Serum pools were prepared from serum samples of patients who were routinely called for hs cTnI measurement and these patients had apparently non-hemolysed, non- lipaemic and non-icteric samples with hs cTnI values <2.3 ng/L and> 100 ng/dL. Two serum pools with 99th percentile upper reference limit (URL) value (17.5 ng/L) and high-risk cut-off value (50 ng/L) were created from these serum pools with appropriate dilutions. Both serum pools were divided into 7 aliquots. Hemolysate was spiked to each aliquata corresponding to H indexes of 100-200-300-400-500-700 and 1000, respectively. The serum rate of hemolysate added to the aliquots did not exceed 5% as suggested by Meitis (16). H indices and hs cTnI levels of all aliquots were measured 2 times within 2 hours and their averages were calculated.

Statistical Analysis

The effect of hemolysis on hs cTnI was calculated according to the formula:

Percent change (%) = (hemolyzed hs cTnI-nonhemolyzed hs cTnI / nonhemolyzed hs cTnI) x 100

The study was performed on randomly selected patient samples after the routine testing was completed, so an informed consent and ethics committee approval were not required. The study was carried out in accordance with the Helsinki Declaration.

RESULTS

In this study, we investigated the interfering effect of hemolysis on the measurement of Beckman Coulter Access hs cTnI at high-risk cut-off concentrations, which are clinically important for the 99th percentile URL and "Rule-in" in the diagnosis of MI. In the study, hemolysis interfered with increasing Hb concentrations to create a negative bias on the initial hs cTnI concentration. Percentage changes of hs cTnIs according to 100-200-300-400-500-700 and 1000 H indices for the 99th percentile group was obtained respectively as -3.8%, -8.5%, -9.6%, -10.8%, and for the high-risk cut-off group it was obtained respectively as -13.1%, -13.7%, -20.4%; -4.3%, -5.7%, -6.2%, -8.8%, -10.3%, -15.4%, -20.0%.

The relationship between the H index and percentage changes of both groups is shown in Figure 1.

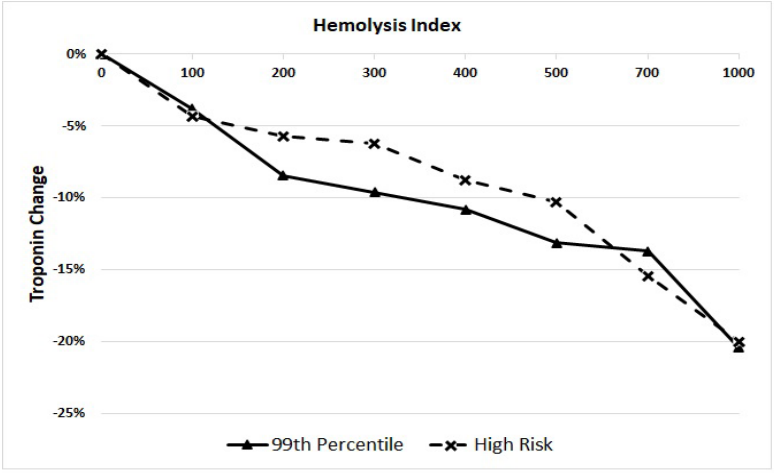

Figure 1. The relationship between the H index and percentage changes of both groups.
Şekil 1. Her iki grubun H indeksi ile yüzde değişimleri arasındaki ilişki.

DISCUSSION

Diagnosing AMI is a complex process that includes patients' clinical findings, ECG evaluation, and laboratory tests. The inadequacy of clinical evaluation and ECG in the diagnosis of NSTEMI has made hs cTn very valuable in both diagnosis and monitoring. Rapid rule-in and rule-out algorithms based on hs cTn concentrations have been developed with serial measurements. In these algorithms, risk assessment for MI is performed on the basis of the measured troponin concentrations and the resulting delta values. This places a great burden on cTns that must be measured reliably, particularly at clinically relevant concentrations. Therefore, in our current study, we chose the 99th percentile URL value defined as myocardial ischemia (4) and the concentration, which is a high-risk cut-off for MI, included in the ESC 2020 guideline (5). In order to evaluate the effect of hemolysis on hs cTnIs at these concentrations, we conducted this study in the index range of 100-1000 H, which is frequently encountered in laboratories. In our study, hemolysis negatively interfered with both hs cTnI concentrations, consistent with increasing free Hb concentrations. The analytical acceptability limit of the difference (bias) between the initial hs cTnI values due to hemolysis interference is ≤ 10% for kit manufacturers (17). In our study, due to hemolysis, this limit was exceeded 10.8% at the 400 H index for the 99th percentile concentration and 10.3% at the 500 H index for the high-risk cut-off concentration. In AMI, a difference of 20% in troponin values is considered clinically significant (18). In our study, it was found that the limit of clinical significance was exceeded at 1000 H index for both troponin concentrations due to hemolysis, 20.4% for the 99th percentile concentration and 20% for the high-risk cut-off concentration.

There are many studies in the literature evaluating the effect of hemolysis on both cTnT and cTnI isoforms of troponin on various device platforms. Bais evaluated the effect of hemolysis on 99th percentile concentrations of both conventional cTnI (Vitros®5600, Ortho-Clinical Diagnostics) and hs cTnT (Roche Elecsys E170). In the study, both troponin measurements showed a 20% change bias around a 150 H index. This change was negative in hs cTnT while it was positive in cTnI (9). Gobeaux et al. evaluated the effect of hemolysis on hs cTnT at concentrations of 26 and 184 ng/L. Hemolysis negatively interfered with both concentrations. While the 20% change, which was considered to be clinically significant, occurred at 250 H index at a concentration of 26 ng/L, this change was 15% for 184 ng/L at

the same H index (10). In another study, Li A. and Brattsand G. added hemolysates with Hb concentrations between 0 and 2 g/L to two different serum pools with hs cTnT concentrations 35 ng/L and 480 ng/L. Similar rates of negative bias were found at both levels (13). Florkowski et al, prepared hemolysates with H indices varying between 120 and 392 to evaluate the effect of hemolysis on both cTnT and cTnI in different device platforms. They spiked these hemolysates into the plasma to measure troponin levels. At the end of the study, negative bias (up to -50%) occurred in hemolysis TnT and hs TnT (Roche Elecsys 2010), while positive bias (+576%) occurred in Vitros ECi-TnI (Ortho-Clinical Diagnostics). In Abbott Architect TnI, up to 15% negative bias occurred at more reasonable levels (11). In another study, Hawkins evaluated the effect of hemolysis on Vitros ECi (Ortho-Clinical Diagnostics) and Accu TnI (Beckman Coulter) measurements. Hemolysates were added to plasma pools of three different TnI concentrations. In the study it was found that hemolysis caused false positives at Vitros ECi TnI concentrations above 0.22 μg/L (12). Lippi et al. used the same device platform as in our study (Beckman Coulter UniCel DxI 800). In order to evaluate the effect of hemolysis on the conventional Accu-TnI test, whole blood samples with EDTA of known troponin values (>20 μg/L) were mechanically traumatized with an insulin injector. Hemolysis had a negative effect on cTnI values. However, the bias formed were not at clinically significant levels until free Hb values reached 1450 mg/dL (14). Ryan et al. Evaluated the susceptibility to hemolysis interference of troponins in the range of 2.3–1126.0 ng/L in the Abbott hs cTnI assay (Architect ci8200 analyzer; Abbott Diagnostics). Non-hemolyzed plasma samples were subjected to hemolysis with free Hb between 0-6.14 g/L. Hemolysis made negative biases on hs cTnI measurements ranging from 4% to 8.3% (15).

In our study, hemolysis decreased the troponin value at the 99th percentile URL by 3.8% and the troponin value at the high-risk cut-off level by 4.3% starting from the 100 H index. Although these differences are within acceptable limits both analytically and clinically, the hs cTnI results obtained may cause myocardial injury and "Rule-in" diagnosis to be missed. However, evaluation of cTn change may be less reliable when even just one sample is hemolyzed in serial measurements in algorithms.

All these findings demonstrate the importance of careful evaluation of hemolysis in troponin measurements. However, none of the immunoassay methods, except those integrated with clinical chemistry, can automatically detect hemolysis due to their nature. Instead, hemolysis is evaluated visually by laboratory workers using color scales (19, 20). However, since visual evaluation can be inadequate to distinguish the limits of hemolysis, it can lead to incorrect sample rejection or acceptance (21).

CONCLUSIONS

Clinical laboratories should be aware of the potential impact of hemolysis interference in troponin measurement. Our study demonstrated the importance of evaluating clinically important tests such as troponin photometrically in terms of hemolysis interference. If photometric evaluation is not possible, we think that it is very important to evaluate patient samples with values close to clinical decision concentrations in terms of hemolysis. We believe that in the future, immunoassay devices should also have technologies that can detect hemolysis.

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