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RESEARCH

DIAGNOSTIC CONCORDANCE BETWEEN NEXT-GENERATION AND HIGH SENSITIVE TROPONIN-I IN ANGINA PECTORIS PATIENTS

(Kesesuaian Diagnostik Troponin-I Next-generation dan High sensitive di Pasien Angina Pectoris)

Erna R Tobing[1], Jusak Nugraha[1], Muhammad Amminuddin[2]

ABSTRAK

Angina pectoris merupakan gejala klinis Sindrom Koroner Akut (SKA) yang mengarah pada penyakit jantung koroner. Sindrom koroner akut terdiri dari Unstable Angina dan Infark Miokard Akut (IMA). Kadar Troponin I (TnI) dapat mendukung penegakkan diagnosis IMA di pasien angina pectoris. Beberapa metode pemeriksaan TnI semakin berkembang diantaranya TnI high sensitive (TnI hs) dan TnI next-generation (TnI ng). Tujuan penelitian ini adalah menganalisis kesesuaian diagnostik antara kadar TnI ng yang diperiksa menggunakan metode Fluorescent Enzyme Transfer Latex (FETL) [Alere Triage MeterPro®] dan TnI hs dengan metode Chemiluminescent Immunoassay (CLEIA) [Mitsubishi PathFast®] di pasien angina pectoris. Penelitian dilaksanakan di RSUD Dr.Soetomo Surabaya masa waktu Maret-Juli 2016 dengan rancangan penelitian potong lintang. Sebanyak 82 subjek penelitian dengan gejala angina pectoris diperiksakan kadar Troponin-I menggunakan kedua metode. Subjek penelitian sebanyak 44% didiagnosis SKA, dan 56% non SKA. Nilai kesesuaian koefisien kappa antara TnI ng dan TnI hs di pasien angina pectoris adalah 0,738 (p<0,01). Kepekaan dan kekhasan TnI ng terhadap TnI hs untuk diagnosis IMA dengan cut off 0,02 ng/mL adalah 94% dan 78%. Analisis kenasaban antara kadar TnI ng dan TnI hs dengan koefisien kenasaban Spearman rho (ρ) adalah 0,826 (p<0,01). Terdapat kesesuaian diagnostik antara TnI ng dan TnI hs di pasien angina pectoris. Kedua metode pemeriksaan TnI dapat digunakan untuk membantu menegakkan diagnosis di pasien angina pectoris. Penelitian lebih lanjut diperlukan untuk mengetahui nilai prognosis TnI.

Kata kunci: *Troponin I, angina pectoris, SKA, FELT, CLEIA*

ABSTRACT

Angina pectoris is a symptom of Acute Coronary Syndrome (ACS) leading to coronary heart disease, consisting of Unstable Angina (UA) and Acute Myocardial Infarction (AMI). Troponin I (TnI) level can help to establish the AMI diagnosis in angina pectoris patients. Some methods of TnI assay have been developed, such as high sensitive TnI (hsTnI) and next-generation TnI (ngTnI). The aim of this study was to analyze the diagnostic concordance between ngTnI using Fluorescent Energy Transfer Latex (FETL) method [Alere Triage MeterPro®] and hsTnI level using Chemiluminescent Enzyme Immunoassay (CLEIA) method [Mitsubishi Pathfast] in angina pectoris patients. This was a cross-sectional study done in the Dr. Soetomo Hospital Surabaya in March-July 2016. The 82 patients with angina pectoris were examined for TnI level simultaneously with both methods. Forty-four percent of subjects were diagnosed as ACS and 56% were non-ACS. The concordance value between ngTnI and hsTnI results in ACS patients was 0.738 (p<0.01). The sensitivity and specificity of ngTnI to hsTnI with a cut off 0.02 ng/mL were 94% and 78%. Correlation analysis between ngTnI and hsTnI by Spearman test revealed a correlation coefficient rho (ρ) = 0.826 (p <0.01). There was a diagnostic concordance between ngTnI and hsTnI in angina pectoris patients. Both TnI assays can be used for establishing the diagnosis in angina pectoris patients. Further research is needed for analyzing the prognostic value of TnI.

Key words: Troponin I, angina pectoris, ACS, FETL, CLEIA

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INTRODUCTION

Angina pectoris is the primary symptom of Acute Coronary Syndrome (ACS) leading to Coronary Heart Disease (CHD).[1] Pathophysiology of ACS begins with the formation of atherosclerosis in the coronary arteries that provide vascularization to the heart muscle. The presence of atherosclerosis causes vasoconstriction and thrombus formation of coronary arteries which can disturb the myocardial perfusion.[2] Ischemia and necrosis of the myocardial muscle causes chest pain, changes in the myocardial electricity and cardiac protein secretion. Acute coronary syndrome consists of Unstable Angina (UA) and Acute Myocardial Infarction (AMI), which includes Non-ST-segment Elevation Myocardial Infarction (NSTEMI) and ST-segment Elevation Myocardial Infarction (STEMI).[3] These diseases can be found mostly in 40-60 years of age, more common in males than females.[4] High rate of morbidity and mortality in ACS patients is due to AMI. Acute myocardial infarction is predicted to be the first common killer worldwide in 2020 which amounts to 36% of all deaths.[5]

The importance of establishing the diagnosis of AMI in ACS patients is a challenge for clinicians to reduce the disability and mortality due to AMI in ACS patients. AMI is established when at least two of the three criteria are met, the following criteria, such as: specific chest pain (angina pectoris); changes in electrocardiography (ECG) pattern and an increase in cardiac biomarkers.[6] Myocardial biomarker assays have an important role in diagnosing ACS especially in patients with nonspecific symptoms and ECG. TnI is the most recommended biomarker because of its better sensitivity and specificity among other biomarkers. Troponin I assay method has been developed to give rapid, accurate and precise results. Several methods of troponin I assay give some sort of parameters of troponin I based on the sensitivity like low sensitive, moderate/contemporary sensitive and high sensitive troponin I (hsTnI). Assay method with hsTnI results can detect a very low elevated level of troponin I. One hs TnI assay method is ChemiLuminescent Enzyme ImmunoAssay method (CLEIA) examined by Mitsubishi PathFast and is used in the Emergency Department (ED), Dr. Soetomo Hospital, Surabaya. Hs TnI examination generally requires sophisticated equipment with advance electrical installations and laboratory facilities so this may be not available in a tertiary laboratory center.

The need of Troponin I examination for diagnosing AMI rapidly and accurately is also for tertiary health facilities that may not have adequate facilities to provide advanced instruments such as those in the Dr. Soetomo Hospital. Alternative methods for troponin I assay is the Fluorescent Energy Transfer Latex (FETL) method by Alere Triage MeterPro that produces troponin I level named as next-generation troponin I (ngTnI) by the company. Fluorescent Energy Transfer Latex method is said to be able to provide a quite sensitive result of troponin I with a smaller instrument, practical, portable and simpler electrical installation. The diagnostic performance of ngTnI parameter as a diagnostic instrument for the ACS stratification and diagnosing AMI is unknown. The gold standard for TnI examination is patients with AMI, while the reference method for TnI assay is still controversial. Assay methods that produce a hsTnI deemed good enough so that it can be used as a comparison method for ngTnI. The aim of this study was to analyze the diagnostic concordance between ngTnI and hsTnI using the above methods in angina pectoris patients.

METHODS

This study was observational analytical with cross-sectional design. The subjects were all patients who visited the Emergency Department of the Dr. Soetomo Hospital, Surabaya from March to July 2016, with complaints of persistent chest pain for > 20 minutes, felt through to the back or spreading to the shoulders, left side arms, neck, mandible or epigastrium, not localized, not associated with movement and position, not disappearing by resting, did not respond or minimally respond to the administration of drugs such as nitrates, could be accompanied by shortness of breath, cold sweat, nausea, or syncope and met the inclusion criteria. The inclusion criteria included age >30 years, signed an informed consent and have a complete medical record. Patients with sepsis and chronic kidney disease were excluded. All study subjects underwent history taking, ECG, physical and laboratory examination.

Troponin I level was examined once in the Emergency Department using EDTA Venous blood sample with Alere Triage MeterPro® and Mitsubishi Pathfast®. Diagnosis of ACS was established by a cardiologist based on criteria of the World Health Organisation (WHO) minimally fulfilling 2 from 3 criteria such as: specific chest pain; electrocardiography (ECG) changes showing patterns such as ST elevation or Q wave; increasing myocardial biomarkers such as CKMB, Troponin I, Troponin T and myoglobin.

The level of the TnI was checked once when the patients were in the ED using Alere Triage MeterPro® and Mitsubishi PathFast®. Performance characteristics of the troponin I examination was shown in Table 1. Next-generation troponin I was the result of examining troponin I using Alere Triage MeterPro®. The assay method was FETL where an analyte in the sample will bind to fluorescently conjugated antibodies and flow in the test device by capillarity. The test device was inserted into the Triage MeterPro and the fluorescent signal was measured within 20 minutes. High sensitive troponin I was the result of examining troponin I using Mitsubishi PathFast®. The high sensitive assay used CLEIA method where the sample was inserted into a reagents cartridge and the analyte will bind the alkaline phosphatase conjugated antibodies that were bound to the magnetic particles. The immune complex formed will be separated from the unbound antibodies by the magnetic field. Chemiluminescence substrate was added and the alkaline phosphatase enzyme from the immune complex will degrade the substrate then produce luminescence light which will be detected by the Pathfast appliance and the result was converted to the level of the troponin I.

The results of an examination of the troponin I was also compared between diagnosis of AMI and non-AMI by a cardiologist. Diagnosis of AMI was established based on the criteria according to the World Health Organization (WHO) which met at least 2 of 3 the following criteria: specific chest pain; changes ECG pattern such as ST-segment elevation or Q waves; (3) Increasing cardiac biomarkers such as CKMB, Troponin I, Troponin T and myoglobin.

Statistical metrics were calculated by using SPSS 9.3. The diagnostic concordance was evaluated by Kappa Cohen test. Sensitivity and specificity were calculated at a clinical cutpoint defined as the 99th percentile of concentrations from the normal reference population. This value was 0.02 ng/mL for Triage TnI and PathFast, based on the manufacturer's recommendations.[7,8] The degree of analytical correlation was tested in samples with detectable troponin levels between the Triage and PathFast. The correlation was evaluated by using Spearman correlation statistics. Only subjects with measured values between the lower and upper limits for both assays in each comparison were included in correlation computations (Triage TnI: lower=0.01 ng/mL, upper=10 ng/mL; PathFast: lower= 0.001 ng/mL, upper= 50 ng/mL).

RESULT & DISCUSSION

The total research samples from 82 subjects consisted of 62 (75.6%) males and 31 (24.4%) females aged 39-82 years with an average age of 57 (SD=10 year). The results of this study showed that 56% subjects were diagnosed as non-ACS and 44% were ACS consisting of STEMI 34% and NSTEMI 55%. ACS patients consisted of 86.11% males and 13.88% females. The value of diagnostic concordance (kappa) between TnI ng and TnI hs on suspected ACS patients was 0.738 ($p<0.01$) meaning that there was a good concordance between the results of the TnI ng and TnI hs. The obtained value of the diagnostic concordance in the ACS patients itself was perfect (kappa: 1,000), while in patients with non-

Table 2. Next-generation troponin I result distribution against high sensitive troponin I

ngTnI (Triage)	hsTnI (PathFast)		
	+*	-	Total
+*	47	7	54
-	3	25	28
Total	50	32	82

*Positive defined as TnI ≥0.02 ng/mL [7,8] for Triage and PathFast
Abbreviation : ngTnI, Troponin next-generation, hsTnI, Troponin high sensitive

Table 1. Assay performance characteristic

	Alere Triage (TnI ng)[7]	PathFast (TnI hs)[8]
LOD	N/A (Limit of blank, 0.01 ng/mL)	0.008 ng/mL
LOQ	0.02 ng/mL	0.01 ng/mL
Reportable range	0.01-10 ng/mL	0.01 – 50 ng/mL
99th percentile	0.02 ng/mL	0.029 ng/mL
CV	16.7% at 0.06 ng/mL and 11% at 5 ng/mL	6.1% at 0.02 ng/mL and 3.9% at 2.51 ng/mL
CV at 99th percentile	<20 %	5%

Abbreviations: N/A, Not Available; LOD, Level of Detection; LOQ, Level of Quantitation; CV, Coefficient Variation; TnI ng, Troponin I next-generation; TnI hs, Troponin I high sensitive

Table 3. TnI ng and TnI hs data result in distribution based on diagnosis

Diagnosis	ngTnI (Triage)			hsTnI (PathFast)		
	+*	-	Total	+*	-	Total
Non-AMI	22	28	50	18	32	50
AMI	32	0	32	32	0	32
Total	54	28	82	50	32	82

* Positive defined as TnI ≥0.02 ng/mL for Triage and PathFast

Abbreviation : AMI, Acute Myocardial Infarction; ng TnI, Troponin next-generation; hs TnI, Troponin high sensitive

ACS there were still differences between the results of the next-generation and high sensitive troponin I. Table 2 showed all subject data results from the ngTnI against hsTnI. The sensitivity and specificity of next-generation TnI against the hs TnI was 94% and 78%, respectively. Table 3 showed the ngTnI and hsTnI data result distribution based on AMI and non-AMI patients. Correlation analysis between ngTnI level and hsTnI by Spearman test revealed a correlation coefficient rho (ρ) = 0.826 (p <0.01).

Non-AMI patients consisted of patients with UA and non-ACS. The positive troponin I results was found not only in patients with AMI, but also in patients with non-ACS including chronic heart failure, stable coronary disease, abnormal heart rhythm (atrial fibrillation, supraventricular tachycardia, bradycardia), cardiomyopathy, pericardial effusion post CABG, diabetes mellitus, HIV infection and chronic lung disease. Similar results were found in the study of Khan et al[9] where troponin I was also found in patients with diagnosis of hypertrophic obstructive, post resuscitation measures of the heart, HIV, central nervous system disease, pulmonary disease, chronic heart failure and hormonal disorders.[9] Troponin I secretion in non-ACS patients with diagnosis of cardiovascular disease was associated with the myocardial damage process. Myocardial necrosis was not only caused by myocardial infarction, but also other cardiovascular disease. The myocardial necrosis whatever the cause can elevate troponin I in the circulation.[9] Mechanism that can explain the elevated troponin I in non-ACS patients with non-cardiovascular disease is the process of systemic organ failure in diseases including heart and also one theory that said that there was a non-specific component like acute phase protein which can bind to the capture antibodies.[9] These theories associating troponin I elevation outside in AMI was still a speculation, while the true mechanism was not yet clear.

A study of Collison et al[10] revealed that troponin I had a very high diagnostic sensitivity for AMI, while the specificity was varied between 46-98%.[10] The specificity was affected by other conditions other than IMA. The study of Carlton et al[11] revealed that the sensitivity of high sensitive TnI with a cut off 0.12 ng/mL was 99% and the negative predictive value was 99.5%, while the study of Neuman et al[12] revealed that the sensitivity of high sensitive TnI with cut off 0.06 ng/mL was 99.8% and the positive predictive value was 82.8%.[11,12] A study by Peacock et al[13] revealed that the sensitivity and specificity of Alere Triage troponin I result was 79.3% and 91.1%; while the high sensitive TnI using Mitsubishi PathFast was 76.7% and 96.8%.[13] Differences in results can be caused by the differences in the methods of examination, the value of the cut off used and differences in research methods such as sample size, sampling method, blood sampling time, etc. A study of Khan et al[9] concluded that troponin I was not a specific cardiac biomarker for AMI, although elevation of troponin I level in non-ACS patients was usually below the AMI patients.[9]

Correlation analysis between the next-generation TnI and high sensitive TnI levels was calculated by Spearman rho coefficient correlation (ρ) and the result was 0.826 (p<0.01). This meant there was a strong positive correlation between next-generation TnI g and high sensitive TnI levels. The result was similar with the study of Peacock et al[13] that revealed that the Spearman Rho coefficient correlation between the next-generation TnI and high sensitive TnI was 0.85.[13] The correlation values were produced by analyzing the quantitative data TnI level that can be detected in each appliances. Some of the results outside the range of the detection of the appliance could not be analyzed and could give influence on the computing correlation coefficient. The amount of detection of the appliance gave limitations in data usage levels of the TnI. The use of the data level may be more useful for monitoring travel diseases with a serial examination compared with a single examination. The limitations of this research were difficulties to determine the onset of chest pain. The value of the correlation is

Table 5. Distribution of troponin I level in the detectable level range

Diagnosis	ngTnI (Triage)* n=63	hsTnI (PathFast)** n=80	Total subject n=82
AMI			
STEMI	6	11	12
NSTEMI	15	19	20
Non-AMI			
UA	3	4	4
Non ACS	39	46	46

* Detection range ngTnI : 0,01-10 ng/mL

** Detection range hsTnI: 0,01-50 ng/mL

AMI: Acute Myocardial Infarction; ngTnI*:* next-generation Troponin I, hsTnI: high sensitive Troponin I*;* UA: Unstable Angina*;* ACS: Acute Coronary Syndrome; STEMI: ST Elevation Myocardial Infarction*;* NSTEMI: Non-ST Elevation Myocardial Infarction.

produced by analyzing the quantitative data in the form of TnI level that could be detected in each of the appliances. Some of the results outside the range of the detection by the appliance could not be analyzed and could give the influence on the computing correlation coefficient. Table 5 displayed the amount of data quantitatively analyzed statistically to obtain a correlation coefficient.

The concordance value between ngTnI and hsTnI in angina pectoris patients was 0.738 ($p<0.01$) showing that the ngTnI, a contemporary troponin I Point of Care (POC), was statistical equivalent with the high sensitive Troponin I. POCT is known easy to be used at bedside patients with small and portable instruments. The benefits can be felt by the angina patients especially in ED and ICU where the decision of diagnosing ACS can be made rapidly to reduce the complication and decrease the number of accidental deaths due to delays in the management of patients.[14] The other method using Mitsubishi Pathfast, a high sensitive troponin I assay, can be used as confirmatory tools of the contemporary method in the case of a very low troponin I level in patient with angina pectoris. As shown in Table 1, the Coefficient Variation of hsTnI using Mitsubishi Pathfast is less than ngTnI using Alere Triage so the hsTnI method has a better precision than the ngTnI. In the case of the angina pectoris patients with an early attack so that the level of TnI still low, or patients in observation of TnI level serially, the hsTnI is more recommended to be used. Some other studies mentioned that POCT will reduce the clinical decision-making time between 1-2 hours after admission to the hospital.[15,16]

CONCLUSION AND SUGGESTION

This study concluded that there was a diagnostic concordance between ngTnI and hsTnI in angina pectoris patients. Both TnI assays showed a good value for helping to establish the diagnosis of ACS patients. Further research is needed for analyzing the prognostic value of TnI.

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