Usefulness of the delta neutrophil index to predict 30-day mortality in patients with ST segment elevation myocardial infarction

Taeyoung Kong

Department of Medicine

The Graduate School, Yonsei University

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Directed by Professor Incheol Park

The Master's Thesis submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medical Science.

Taeyoung Kong

December 2016

This certifies that the Master's Thesis of
Taeyoung Kong is approved.

Thesis Supervisor : Incheol Park

Thesis Committee Member#1 : Sung Phil Chung

Thesis Committee Member#2 : Jong Beack Lim

The Graduate School
Yonsei University

December 2016

ACKNOWLEDGEMENTS

First, I want to appreciate my supervisor Prof. Incheol Park for his great lessons and guidance in conducting this study. Without his kind consideration, I could not finish the Master's thesis. I would like to express great appreciation to my Prof. Sung Phil Chung, who looks after me warmly. My deepest appreciation also goes to Prof. Yoo Seok Park. He has given me great support to lead me steps in the path of righteousness. And, I want to express my deepest appreciation to Prof. Je Sung You, who gave me a passionate teaching in research and life, as a mentor. I would also like to appreciate Prof. Jong Beack Lim.

Lastly, I would like to show my gratitude to my fellow emergency medicine professors of Yonsei university college of medicine. I could successfully perform study and write the Master's thesis thanks to their warm consideration.

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ABSTRACT

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Taeyoung Kong

Department of Medicine
The Graduate School, Yonsei University

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Objective

To evaluate the association between the delta neutrophil index (DNI), reflecting immature granulocytes, and severity of ST-elevation myocardial infarction (STEMI). We evaluated the DNI to determine its significance as a prognostic marker for early mortality in patients with STEMI who underwent reperfusion.

Design

A retrospective, observational cohort study.

Setting

A single 2000-bed tertiary level teaching hospital that attends to 85,000 patients in the emergency department annually.

Patients

We included 619 patients diagnosed with STEMI who underwent primary

percutaneous coronary intervention (pPCI) between January 1, 2011 and May 30, 2016.

Interventions

None.

Measurements and Main Results

Demographic and clinical data were collected from consecutive patients prospectively integrated in the critical pathway program for STEMI. DNI was collected on admission to the emergency department, immediately after reperfusion (time-I; within 2 h of pPCI), and 24 h after admission (time-24). The clinical outcome was 30-day mortality. A total of 619 STEMI patients were enrolled in this study. According to multivariable Cox proportional hazard model, higher ratios at time-I (hazard ratio [HR], 1.065; 95% confidence interval [CI]: 1.031–1.101; $p<0.001$), and time-24 (HR, 1.067; 95% CI: 1.038–1.098; $p<0.001$) were significant risk factors for 30-days mortality. Among patients with STEMI who underwent reperfusion, DNI values >4.6 % at time-I (HR, 4.57; 95% CI: 2.493–8.378; $p<0.001$) and >4.8% at time-24 (HR, 6.049; 95% CI: 3.371–10.854; $p<0.001$) were associated with an increased risk of 30-day mortality among patients with STEMI who underwent pPCI.

Conclusions

DNI values, obtained without an additional burden of cost or time, can be measured rapidly and simply after admission. Higher DNI levels independently predict 30-day mortality in patients with acute STEMI after pPCI.

Key words: ST-elevation myocardial infarction, Delta neutrophil index, Mortality

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The Graduate School, Yonsei University

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I. INTRODUCTION

Acute myocardial infarction (AMI) remains a major cause of mortality and morbidity worldwide.[1] Recent improvements in critical care medicine, such as the availability of reperfusion therapies and subsequent comprehensive management, have led to a considerable reduction in short- and long-term mortality.[2,3] However, patients with ST segment elevation (STEMI) on the presenting electrocardiogram (ECG) remain at increased risk of mortality and serious morbidity if they survive the initial ischemic event.[4] It is widely accepted that accurate and rapid assessment of severity critically affects treatment and prognosis of patients with STEMI.[1] Many studies have attempted to develop cardiac-specific markers or risk scoring systems to identify patients at increased risk and to provide prognostic information.[5] Therefore, prognostic factors that can be measured rapidly, easily, and accurately in emergencies are needed to

assess severity in patients with AMI. Recently, the role of inflammatory markers in severity assessment in early stage of STEMI has been of interest. In AMI, the progress of atherosclerosis and thrombosis are closely associated with inflammation.[6] Early ischemic injury leads to an extreme inflammatory response; AMI also triggers an acute inflammatory response, which serves to repair injured heart muscle.[7] Although primary percutaneous coronary intervention (pPCI) restores patency of epicardial coronary arteries, reperfusion injury by tissue edema, endothelial disruption, and inflammation worsens ischemia-related injury. Moreover, failure of microvascular perfusion, known as no-reflow (NR), results in optimal myocardial perfusion not being restored.[8] PCI itself is a strong additional inflammatory stimulus and may cause acute systemic inflammatory responses leading to post-PCI complications.[9] Despite much experimental and clinical evidence of the association between inflammation and adverse outcomes, no specific inflammatory biomarkers are currently used in the standard management of patients with STEMI.[4,10] The immature-to-total granulocyte ratio or neutrophil band count is increased in infection, ischemia, and systemic inflammation, reflecting the presence of immature granulocytes.[11-13] This is a practical marker of local and systemic inflammation.[11,13,14] The specific automated blood cell analyzer—a recent technological advance—allows rapid determination of the delta neutrophil index (DNI) along with the complete blood count (CBC). The DNI reflects the fraction of circulating immature neutrophils.[11-13] To the best of our knowledge, this is the first study to evaluate the association between the DNI and the severity of STEMI in a clinical setting. We evaluated the DNI to determine its significance as a prognostic marker of early mortality in patients with STEMI who underwent pPCI.

II. MATERIALS AND METHODS

1. Study population

This retrospective, observational cohort study was conducted between January 1, 2011 and May 30, 2016 at a single tertiary academic hospital that attends to 85,000 patients in the emergency department (ED) annually. The study was reviewed and approved by the institutional review board of Yonsei University Health System (No. 3-2015-0140).

In 2007, a multidisciplinary critical pathway based on a computerized provider order entry (CPOE) system, known as FIRST, was implemented in the Yonsei University College of Medicine-affiliated Severance Hospitals. Our critical pathway for STEMI management was designed to reduce unnecessary in-hospital time delays through a CPOE-based alert system, short message service, and simple standing orders through the activation stage. The present study included consecutive patients who were prospectively integrated into the FIRST critical pathway program, analyzing those admitted with STEMI who underwent pPCI between January 1, 2011 and May 30, 2016.

Upon arrival of a patient to the ED, the physicians, nurses, and emergency medical technicians in the triage area identified candidates for the FIRST program as soon as possible according to pre-determined protocols. Preferentially, patients with typical or non-specific symptoms of suspected AMI (e.g., chest pain, epigastric pain, syncope, dizziness, vertigo, shock, dyspnea, nausea, and/or vomiting) were examined. Simultaneously, a 12-lead ECG was performed in the triage area. The criteria for critical pathway activation were based on the ECG criteria in the standard STEMI guidelines and the duration since the onset of chest pain [15]. ST-segment elevation in the ECG criteria was defined as J-point elevation on two or more contiguous leads, with a threshold of >2 mm in the precordial leads or >1 mm in other leads, and a new or presumed new left bundle branch

block. When a patient had at least one predetermined ECG warning criterion for ST-segment elevation on ED arrival, within 12 h of the onset of symptoms, the triage ED physician activated the FIRST program by selecting the activation icon on the order entry window.[15] Once activated, the on-call cardiologist was consulted. The on-call cardiologist immediately assessed the patient and applied standard treatment in accordance with the guideline of the American College of Cardiology Foundation/American Heart Association (ACCF/AHA). Coronary angiography and PCI were conducted using standard protocols and guidelines.

We excluded patients lost to follow-up; treated without pPCI; with stress induced cardiomyopathy; with "do not attempt resuscitation" status; or with comorbid conditions such as hematologic malignancy, chronic inflammation, current infection, and use of immunosuppressive agents or chemotherapy within the 14 days before ED admission.

2. Data collection

We examined data on patients' demographics, laboratory test results (including cardiac enzymes), PCI findings, and left ventricular ejection fraction based on a predetermined protocol. We also evaluated data on Killip classification, Global Registry of Acute Coronary Events (GRACE) score, and the door-to-balloon time interval. The DNI for each patient was determined at time 0 (immediately on ED admission), time I (within 2 h post-pPCI), and time 24 (24 h post-admission). Venous blood was collected in ethylenediaminetetraacetic (EDTA) containing vacutainers on presentation to the ED and at multiple time points (within 2 h of reperfusion, and 24 h and 48 h after ED admission) for measurement of DNI, using the same type of hematology analyzer used for the analysis of complete blood count (CBC).

3. DNI and other blood sample measurements

A CBC, comprising DNI, white blood cell count (WBC), hemoglobin level, and platelet count, was analyzed by an automated blood cell analyzer (ADVIA 2120; Siemens, Forchheim, Germany). The specific analyzers comprise two independent WBC analysis methods using flow cytometric principles. First, the optical system based on the cytochemical myeloperoxidase tungsten-halogen channel measured and differentiated granulocytes, lymphocytes, and monocytes based on size and myeloperoxidase content staining intensity.[12,13] Second, the optical system, using the lobularity/nuclear density channel laser-diode, calculated and classified cell types with respect to lobularity/nuclear density and size.[12,13] DNI was then calculated by subtracting the fraction of mature polymorphonuclear neutrophils from the sum of the myeloperoxidase-reactive cells, detecting circulating immature granulocytes as the leukocyte sub fraction.[12,13] In other words, DNI was obtained using the following formula: DNI = (neutrophil sub-fraction + eosinophil sub-fraction) − (neutrophil sub-fraction).[12,16] Other laboratory tests conducted at the time of ED admission included determination of blood urea nitrogen (BUN), creatinine, alanine transaminase, high-sensitivity C-reactive protein (hs-CRP), creatine kinase (CK), CK-MB, Troponin-T (Tn-T), N-terminal pro B-type natriuretic peptide (NT-proBNP), and albumin levels, assessed using an automated chemistry analyzer.

4. Clinical outcomes

The clinical outcome of interest was 30-day mortality.

5. Statistical analysis

Demographic and clinical data are presented as medians (interquartile ranges [IQRs]), means ± standard deviations (SDs), and percentages or frequencies as appropriate. Continuous variables were compared using a two-sample *t*-test or

the Mann–Whitney U-test. Categorical variables were compared using the χ^2 test or Fisher's exact test. We estimated significant differences between groups over time using a linear mixed model and repeated measures covariance pattern with unstructured covariance within patients. Two fixed effects were included to address the clinical effect (level: survival and death) and time effect (time: DNI performed at on admission, immediately after pPCI, and 24 h and 48 h after ED admission). Differences in clinical effect over time were analyzed according to clinical effect × time. We performed univariable analyses to evaluate relationships among demographic characteristics and clinical data. We also identified promising independent factors predictive of 30-day mortality by considering time-to-event data in patients with STEMI undergoing pPCI using a multivariable Cox proportional hazard regression analysis that integrated the major covariates (variables with a $p < 0.05$) identified in our univariable analyses. The results are expressed as hazard ratios (HRs) and 95% confidence intervals (CIs).

To investigate the additional predictive power of DNI at each time point, we calculated Harrell's C index for each Cox model.[17,18] To calculate the 95% CI and p-value for Harrell's C index and difference between models, we used a standard bootstrap method with resampling 1,000 times.[17] We also assessed the continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI) at the median follow-up time point (5 days) to assess the improvement in performance of the survival model with DNI.[17] We compared Harrell's C index to assess at which time point the DNI provided the better prognostic value. Kaplan-Meier survival curves were created using 30-day mortality data, and groups were compared using the log-rank test. Although previous studies estimated cut-off values based only on events, we estimated optimal cut-off values for the dichotomization of the clinical outcome variable based on time-to-event data using the technique devised by Contal and O'Quigley. The optimal cut-off point was selected by maximizing the HR. Statistical analyses

were performed using SAS, version 9.2 (SAS Institute Inc., Cary, NC); R software, version 3.2.5 for Windows (the R foundation for statistical computing, Vienna, Austria; http://www.R-project.org/); and MedCalc, version 12.7.0 (MedCalc Software, Ostend, Belgium). A p-value <0.05 was considered significant.

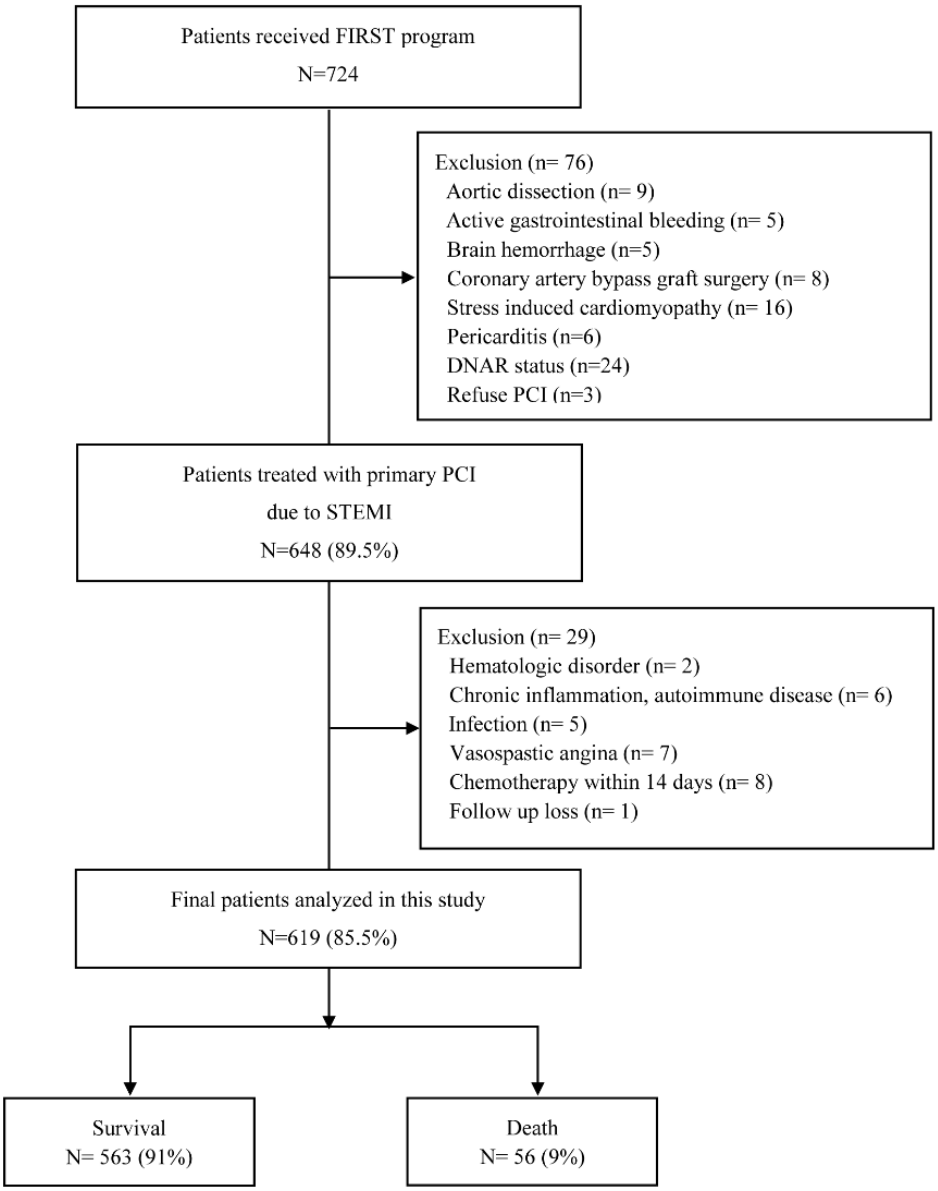

Figure 1. Flow diagram of patient enrolment

III. RESULTS

Figure 1 shows the enrolment and clinical outcome data for patients with STEMI registered in the FIRST program. 619 (85.5%) were enrolled in this study. Their baseline characteristics and clinical data are presented in Table 1. The mean DNI values immediately after pPCI and 24-h post-admission were significantly higher in the non-survival group who died within 30 days than in the survival group at admission (Table 1). A linear-mixed model revealed significant differences in DNI values between patients grouped according to 30-day survival ($p<0.001$ for all; Figure 2). The univariable Cox regression analysis revealed significant differences in DNI values at times-I and 24 between the non-survival and survival groups (Table 2).

The multivariable Cox proportional hazard model further confirmed the association between increased DNI values at times-I and 24 and an increased risk of 30-day mortality among patients with STEMI who underwent pPCI (Table 3). C index of each Cox model was assessed to evaluate its discriminatory usefulness. The C-index of models 1, 2, 3, and 4 were 0.879, 0.883, 0.888, and 0.892, respectively. Despite relatively higher C-index, adding DNI over time to survival models revealed an increased trend of C-index that did not reach statistical significance. However, the addition of DNI yielded significantly positive values of IDI for DNI at times I and 24. Continuous NRI was also positive, which was significant for DNI at time-I and showed an increased trend for DNI at time-24. In comparison, the C-index of DNI at times-I and 24 were statistically superior to WBC, neutrophil count, and percentage of neutrophils. Although the C-index of DNI at time-0 was less than that of Tn-T and NT-proBNP at admission, DNI at time-I was not significantly inferior to CK-MB, Tn-T, and NT-proBNP at admission. DNI at time-24 was better at predicting 30-day mortality than CK-MB or CRP measured 24 h post-admission (Figure 4).

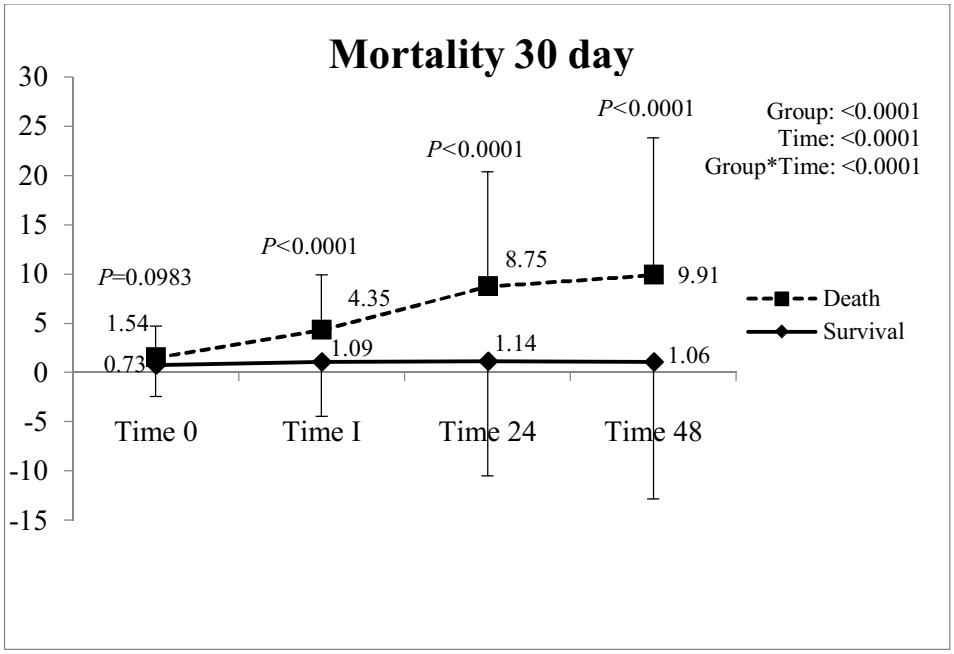

Figure 2. Linear mixed model of the delta neutrophil index

To estimate the optimal cut-off values based on time-to-event data, Kaplan-Meier curves of 30-day mortality were generated from the DNI values at times 0, I, and 24. These DNI values were also independent predictors of clinical outcomes within 30 days post-pPCI. Specifically, an increased risk of 30-day mortality was observed among patients with an increased DNI at each time point after ED admission. In slight contrast to the results described above, the log-rank test indicated that the optimal DNI cut-off values for 30-day mortality predictions were 1.0% at time-0 (p=0.03), 4.6% at time-I (p<0.001), and 4.8% at time-24 (p<0.001). Further analysis of these cut-off values using the Contal and O'Quigley technique indicated that DNI values >4.6% at time-I (HR, 4.57; 95% CI: 2.493–8.378; p<0.001) and >4.8% at time-24 (HR, 6.049; 95% CI: 3.371–

10.854; p<0.001) were associated with an increased risk of 30-day mortality among patients with STEMI who underwent pPCI (Figure 3).

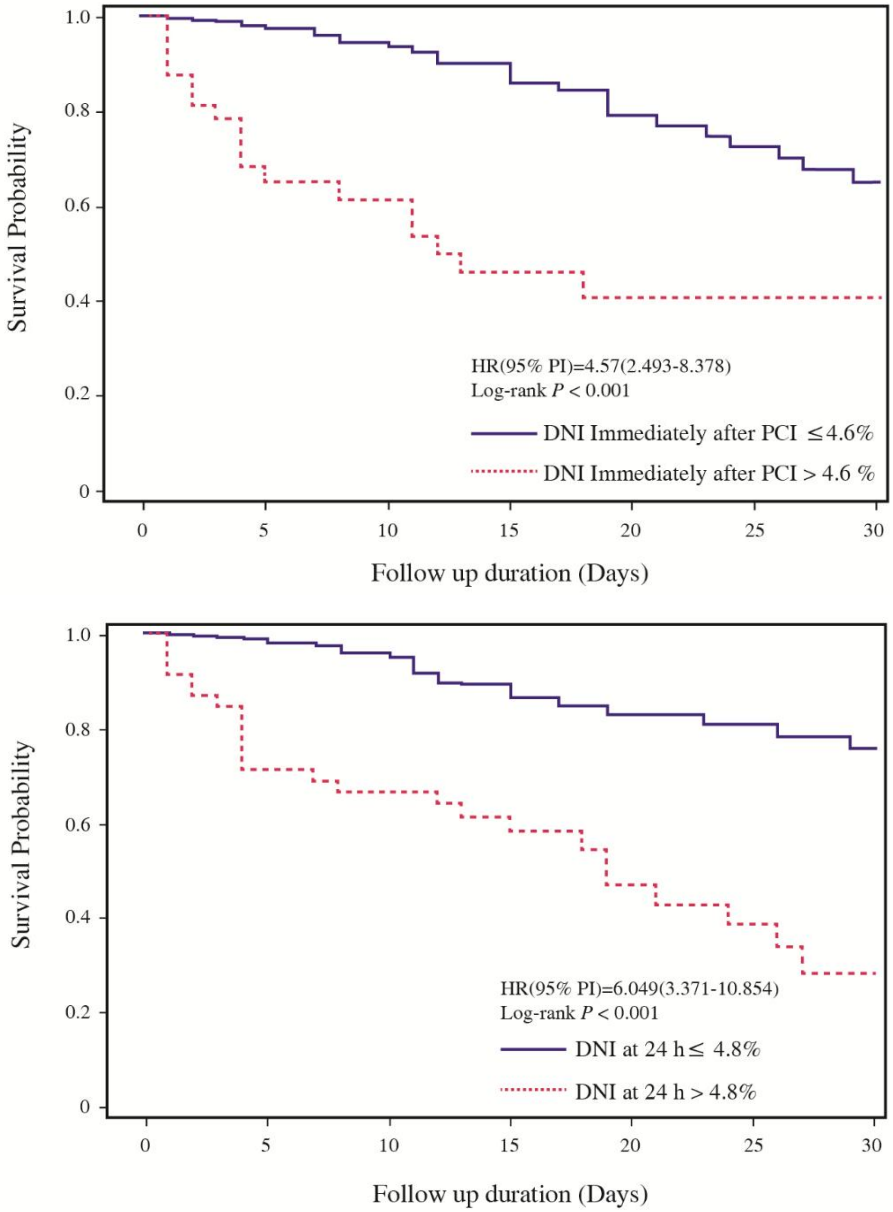

Figure 3. Delta neutrophil index (DNI) as a predictor of 30-day mortality

Table 1. Clinical characteristics of patients stratified according to 30-day mortality. GRACE, Global Registry of Acute Coronary Events; NT pro BNP, N-terminal pro-brain natriuretic peptide; PCI, percutaneous coronary intervention; TIMI, thrombolysis in myocardial infarction; DNI, delta neutrophil index. Data are expressed as mean ± SD or number (percentage).

Variables	Total (N=619)	30-day mortality		*p*-value
		Survivors (N=563)	Death (N=56)	
Male Sex (N, %)	488(78.8)	450(79.9)	38(67.8)	0.035*
Body mass index (kg/m²)	24.1±3.4	24.2±3.3	23.8±3.7	0.434
Smoker (N, %)	270(43.6)	238(42.2)	32(57.1)	0.032*
GRACE score	169.4±46.1	164.2±43.7	223.0±34.1	<.001*
Age	62.9±13.1	62.2±13.1	69.8±11.2	<.001*
Heart rate (/min)	79.0±25.8	78.8±23.6	80.8±42.4	0.725
Systolic blood pressure (mmHg)	126.1±39.5	128.9±37.1	97.7±50.2	<.001*
Creatinine (mg/dL)	1.1±1	1.1±0.9	1.6±0.9	0.001*
Creatine kinase MB (mcg/L)	30.0±70.0	25.8±58.6	72.3±134.3	0.013*
Troponin T	671.2±1595.7	543.0±1311.9	1958.5±3026.1	0.001*
Arrest on admission	18(2.91)	11(1.95)	7(12.5)	0.001*
Killip class at admission				<.001*
Ⅰ	249(40.2)	244(43.3)	5(8.9)	
Ⅱ	143(23.1)	141(25.0)	2(3.5)	
Ⅲ	97(15.6)	82(14.5)	15(26.7)	
Ⅳ	130(21)	96(17.0)	34(60.7)	
Clinical measurements on admission				
Respiratory rate(/min)	16.2±4.0	16.3±3.7	15.0±6.7	0.164
Left ventriclular ejection fraction(%)	44.3±12.3	45.9±11.2	28.3±12.3	<.001*
NT pro BNP	2510±6300	2032±5651	7420±9727	<.001*
White blood cell count(/μL)	11968±10942	11496±3900	16709±34130	0.258
Neutrophil ratio(%)	64.9±17.0	64.8±16.7	66.0±20.1	0.690
Absolute neutrophil count(/μL)	7754±3976	7688±3841	8429±5157	0.304
High sensitivity C reactive protein(mg/L)	15.3±36.6	14.4±36.3	22.9±38.3	0.146
Activated partial thromboplastin time	36.9±33.4	35.9±30.9	47.4±51.4	0.105
Hematocrit(%)	42.5±5.6	42.7±5.4	40.1±7.2	0.011*
Platelet(10^3/μL)	244.3±78.1	246.4±76.8	222.8±88.5	0.031*
Total cholesterol(mg/dL)	187.5±46.6	188.7±46.6	175.5±45.6	0.044*
Triglyceride(mg/dL)	115.8±132.1	117.9±136.3	88.9±47.3	0.002*
Sodium(mmol/L)	139.5±3.6	139.5±3.1	139.5±6.6	0.980
Glucose(mg/dL)	195.7±92.6	190.0±87.6	253.1±119.7	<.001*
Medical History				
Hypertension	308(49.7)	280(49.7)	28(50)	0.970
Diabetes mellitus	173(27.9)	151(26.8)	22(39.3)	0.047*
Chronic obstructive pulmonary disease	10(1.6)	8(1.4)	2(3.6)	0.227
Hyperlipidemia	79(12.7)	74(13.1)	5(8.9)	0.367
History of PCI	72(11.6)	68(12.1)	4(7.1)	0.272
History of coronary artery bypass graft	6(0.9)	5(0.9)	1(1.8)	0.435
Ischemic heart disease	102(16.4)	90(15.9)	12(21.4)	0.295
Heart failure	17(2.7)	14(2.5)	3(5.4)	0.193
Arrhythmia	11(1.7)	10(1.8)	1(1.8)	0.999
Stroke	32(5.1)	24(4.3)	8(14.3)	0.005*
Peripheral arterial occlusive disease	8(1.29)	7(1.2)	1(1.8)	0.534
Malignancy	31(5.0)	26(4.6)	5(8.9)	0.188

Metastatic Cancer	4(0.7)	3(0.5)	1(1.8)	0.316
Chronic kidney disease	23(3.7)	17(3.0)	6(10.7)	0.013*
Chronic liver disease	5(0.8)	4(0.7)	1(1.8)	0.379
Peptic ulcer	3(0.5)	3(0.5)	0(0)	0.999
Major depressive disorder	2(0.3)	2(0.4)	0(0)	0.999
Procedural characteristics				
Door-to-balloon time (min)	56.9±29.6	55.9±29.2	67.1±31.8	0.007*
Narrowed coronary artery				0.154
1 vessel	238(38.5)	221(39.3)	17(30.4)	
2 vessel	205(33.1)	188(33.4)	17(30.4)	
3 vessel	176(28.4)	154(27.4)	22(39.3)	
Infarct-related coronary artery				<.001*
Left anterior descending	324(52.3)	301(53.5)	23(41.1)	
Left circumflex	55(8.9)	46(8.2)	9(16.1)	
Right	208(33.6)	194(34.5)	14(25)	
Left main	32(5.2)	22(3.9)	10(17.9)	
TIMI flow grade pre-PCI (≤1)	141(22.8)	131(23.27)	10(17.9)	0.357
DNI % (at admission)	0.8±1.9	0.7±1.7	1.5±3.2	0.062
DNI % (immediately after PCI)	1.5±3.9	1.1±2.2	5.5±8.9	0.001*
DNI % (24 hours after admission)	1.8±4.6	1.1±2.3	9.3±11.9	<.001*

Table 2. Univariable Cox proportional hazard regression analysis for predictors of 30-day mortality. CI, confidence interval; GRACE, Global Registry of Acute Coronary Events; NT pro BNP, N-terminal pro-brain natriuretic peptide; PCI, percutaneous coronary intervention; TIMI, thrombolysis in myocardial infarction; DNI, delta neutrophil index.

Variables	30-day mortality		*p*-value
	Hazard ratio	95% CI	
Male Sex	0.717	0.408-1.260	0.248
Body mass index	0.982	0.912-1.057	0.624
Smoker	1.554	0.915-2.642	0.103
GRACE_score	1.021	1.014-1.029	<.001*
Age	1.033	1.011-1.056	0.003*
Heart rate	1.004	0.995-1.012	0.391
Systolic blood pressure	0.993	0.987-0.998	0.009*
Creatinine	1.039	0.905-1.192	0.589
Creatine kinase MB	1.004	1.002-1.006	<.001*
Troponin T	1	1-1	<.001*
Arrest on admission	1.775	0.784-4.02	0.169
Killip class at admission			
Ⅰ	1		
Ⅱ	0.658	0.128-3.395	0.617
Ⅲ	4.666	1.673-13.018	0.003*
Ⅳ	6.046	2.29-15.963	<.001*
Clinical measurements on admission			
Respiratory rate	0.979	0.937-1.022	0.332
Left ventricular ejection fraction	0.933	0.914-0.953	<.001*
NT pro BNP	1	1-1	0.011*
White blood cell count	1	1-1	0.002*
Neutrophil ratio	1.007	0.992-1.022	0.348
Absolute neutrophil count	1	1-1	0.375
High sensitivity C reactive protein	1.003	0.997-1.009	0.349
Activated partial thromboplastin time	1.003	0.998-1.009	0.191
Hematocrit	0.973	0.933-1.014	0.195
Platelet	0.997	0.994-1.001	0.136
Total cholesterol	0.998	0.993-1.004	0.587
Triglyceride	0.995	0.989-1.002	0.151
Sodium	1.027	0.955-1.104	0.478
Glucose	1.002	1-1.004	0.132
Medical History			
Hypertension	1.011	0.599-1.708	0.967
Diabetes mellitus	1.132	0.656-1.953	0.657
Chronic obstructive pulmonary disease	1.296	0.314-5.35	0.720
Hyperlipidemia	0.591	0.236-1.482	0.262
History of PCI	0.492	0.178-1.361	0.172
History of coronary artery bypass graft	2.736	0.376-19.928	0.320
Ischemic heart disease	1.022	0.538-1.944	0.946
Heart failure	1.660	0.517-5.323	0.394
Arrhythmia	0.634	0.088-4.595	0.652
Stroke	3.256	1.533-6.915	0.002*
Peripheral arterial occlusive disease	1.073	0.148-7.769	0.944
Any malignancy	1.150	0.456-2.906	0.767

Metastatic Cancer	1.631	0.225-11.843	0.629
Chronic kidney disease	1.571	0.666-3.704	0.302
Chronic liver disease	3.301	0.452-24.092	0.239
Peptic ulcer	0.918	0.055-15.465	0.953
Major depressive disorder	2.085	0.122-35.597	0.612
Procedural characteristics			
Door-to-balloon time	1.005	1-1.011	0.040*
Narrowed coronary artery			
1 vessel	1		
2 vessel	1.134	0.579-2.221	0.715
3 vessel	1.317	0.697-2.488	0.397
Infarct-related coronary artery			
Left anterior descending	1		
Left circumflex	1.979	0.914-4.285	0.083
Right	1.035	0.532-2.015	0.919
Left main	2.413	1.135-5.128	0.022*
TIMI flow grade pre-PCI (≤1)	0.679	0.343-1.346	0.268
DNI (at admission)	1.069	0.988-1.158	0.098
DNI (immediately after PCI)	1.076	1.048-1.106	<.001*
DNI (24hours after admission)	1.078	1.057-1.098	<.001*

IV. DISCUSSION

In this study of patients with STEMI who underwent pPCI, we found that DNI was a significant independent predictor of 30-day mortality. Specifically, we found that DNI values >4.6% immediately (within 2 h) after pPCI and >4.8% at 24 h post-admission could significantly predict 30-day mortality in this group of patients. Hence, we propose the use of these DNI values—that can be determined rapidly, easily, and inexpensively—to assess severity in such patients.

In the pathogenesis of acute coronary syndromes, inflammation exerts on important effect on initiation and progression of atherosclerosis and contributes to plaque rupture and thrombus development.[19] AMI triggers an acute inflammatory response that is exaggerated, moving from a local myocardial inflammatory process to a systemic inflammatory response.[7] Van Diepen et al. demonstrated that a cumulative increase of individual systemic inflammatory response syndrome (SIRS) criteria was independently associated with increased risk of 90-day clinical outcomes in patients with STEMI.[20] Of the SIRS criteria, only heart rate and WBC count were significantly associated with increased risk of the primary outcome.[20] Heart rate (HR) was more significantly increased than WBC count on admission, whereas WBC count was more significantly associated with outcome than HR at 24 h.[20] In AMI, profound systemic inflammation, caused by dysregulation of the immune system, is associated with increased inflammatory mediators and activation of peripheral leukocytes and neutrophils, or neutrophil subtypes.[7] An intense inflammatory response is activated in the early stage of cardiac ischemic injury; this contributes significantly to ventricular remodeling after AMI.[4] The leukocytosis in STEMI reflects activation and infiltration of neutrophils into the necrotic tissues of ischemia-reperfusion injury. Neutrophils are the critical cells in innate immunity. They mediate tissue damage after ischemia-reperfusion injury.[7] Tamhane et al. demonstrated that neutrophilia >65% in patients with STEMI reflected worse angiographic outcomes, large

infarct size, and increased short-term mortality.[21] Furthermore, the neutrophil count and neutrophil-to-lymphocyte ratio as simple inflammatory biomarkers independently predicted short- and long-term outcomes in patients with AMI.[19,21] In acute coronary syndrome, neutrophils are activated and recruited to the injury sites, where they mediate inflammation.[19] Activated neutrophils damage the myocardium, leading to microvascular obstruction.[22] Neutrophil plugging is the most critical cause of post-PCI major adverse cardiac events.[19,22] In addition, immature granulocytes enter the circulation during infection, stress, or systemic inflammation; SIRS criteria include an increase in the number of immature granulocytes in circulation.[11,23] Van Hout et al. demonstrated that the relative distribution of band neutrophils was significantly increased in peripheral blood after reperfusion in AMI.[22] Altered subset composition and systemic activation of neutrophils reflects the amount of cardiac damage after AMI.[22] Our study also demonstrated that the DNI increased significantly after reperfusion. The value of DNI within 2 h after reperfusion was superior to WBC count, neutrophil count, and percentage of neutrophils for predicting short-term mortality in patients with STEMI.

Despite the importance of neutrophils in STEMI, manual counting of banded neutrophils, which involves a 200-cell manual differential count of a blood smear and is difficult to conduct rapidly after staining, might be inaccurate when compared with DNI, measured using simultaneous 30,000-cell differential count and staining.[24] [12,13] Nahm et al. demonstrated that DNI was strongly correlated with manual immature granulocyte counts and that the automated blood cell analyzer for DNI can overcome the limitations of manual counting of immature granulocytes.[11,12] The present study demonstrated that DNI values over time were significantly increased in the mortality group compared with the survival group; these changes were associated with clinically poor outcomes in patients with STEMI. Our results suggest that patients with STEMI undergoing pPCI should be carefully monitored if the DNI value exceeds 4.6%. Similarly, a previous study

by Yune et al. reported that DNI >8.4% on admission (HR, 3.227; 95% CI, 1.485–6.967; p = 0.001) and DNI >10.5% on day 1 (HR, 3.292; 95% CI, 1.662–6.519; p < 0.001) were associated with increased 30-day mortality in patients surviving out-of-hospital cardiac arrest.[11] The clinical implication of these findings is that increased DNI reflects severity of systemic and sterile inflammation.[11]

In terms of sepsis, a previous study by Park et al. revealed that a DNI >6.5% was a good diagnostic marker of severe sepsis and septic shock within the first 24 h after intensive care unit admission.[13] Considering the significant increase in DNI after pPCI from baseline over time, DNI values after pPCI are able to differentiate those patients with STEMI who have a more severe clinical course. In sepsis, previous studies have proposed clearer mechanisms to explain this rapid and early release of immature granulocytes. In the cases of sterile inflammation, such as AMI or post-resuscitation after out-of-hospital cardiac arrest, the mechanism for increasing immature granulocytes is likely similar to that in sepsis. For example, immune responses to sterile inflammation also induce release of immature granulocytes, a result of the rapid expansion of circulating neutrophils to compensate for a loss of active neutrophils due to the massive consumption and destruction of mature cells in severe inflammation.[23,25-27] In myocardial reperfusion injury, reperfusion induces endothelial dysfunction, which results in vasoconstriction during the first few minutes, while increased leukocyte adhesion and influx result in impaired blood flow.[28] Death of cardiomyocytes can be directly induced by these leukocytes. Endothelium can be indirectly injured by production of reactive oxygen species.[28] In addition, neutrophil paralysis—known as dysregulated neutrophil function—attenuates tissue damage in severe sterile inflammation as a result of impaired migration of neutrophils to the injured site and neutrophil sequestration in remote organs.[25,26] Sauneuf et al. suggested bone marrow exhaustion as a further mechanism by which severe inflammation, induced by ischemia, could lead to a transient failure of regulation of neutrophil release during ischemia and following resuscitation.[27,29] In particular,

hemodynamic instability or persistently severe inflammation, according to an increase in severity of STEMI, may affect critical regulatory mechanisms for neutrophil release from the bone marrow. The DNI after reperfusion may be a promising biomarker for predicting severity and mortality in the early stages of STEMI.

Although AMI can be diagnosed with cardiac troponin and ECG abnormalities based on suggestive symptoms of AMI, many studies have attempted to propose and validate risk stratification systems for identification of patients at high risk of death or with a critical prognosis.[5,6] Among several scoring systems, the GRACE risk score, which includes two biomarkers (serum creatinine and troponin), is widely accepted to estimate prognosis. It is a relatively simple assessment to perform and is effective.[6] Clinically, the risk score is able to differentiate critical patients at high risk and to predict the mortality rate in patients with STEMI in the ED setting.[6,30] They are excessively complicated with respect to early severity, and require serial or multiple measurements to determine severity. Serum biomarkers, such as BNP and hs-CRP, are widely used to identify ischemia-reperfusion injury and prognosis after reperfusion in patients with STEMI, assisting in estimation of infarct size, microvascular obstruction, left-ventricular remodeling, and stratification of risk in patients with AMI.[31,32] Both peak CK-MB and peak troponin I levels were independently shown to be associated with in-hospital mortality.[33] However, the cost effectiveness of risk prediction must be considered in the requirement for serial measurements of cardiac specific markers in the clinical setting.[5] The clinical utility of a biomarker for risk prediction depends on practicability, ease, cost, and reproducibility of the measurement, and the ability to add it to the predictability of existing biomarkers.[34]

Our study revealed that DNI within 2 h post-reperfusion had similar predictability as CK-MB, Tn-T, and BNP for 30-day mortality. In addition, the value of DNI 24 h post-admission was superior to CK-MB and hs-CRP for

predicting 30-day mortality. DNI has the added benefit of being automatically analyzed with the CBC, which is routinely and immediately performed in critically ill patients, without additional time or cost, unlike hs-CRP, CK-MB, and Tn-T. We propose that DNI, by reflecting systemic inflammation, may be a promising marker for assessment of severity in patients with STEMI after pPCI. In future, prospective multicenter studies with a larger number of patients will be needed to validate our findings.

Table 3. Multivariable Cox proportional hazard regression analysis for predictors of 30-day mortality. CI, confidence interval; GRACE, Global Registry of Acute Coronary Events; NT pro BNP, N-terminal pro-brain natriuretic peptide; DNI, delta neutrophil index; PCI, percutaneous coronary intervention.

Variable	DNI (at admission)		DNI (immediately after PCI)		DNI (24hours after admission)	
	Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
GRACE score	1.018(1.007-1.028)	**<.001***	1.018(1.008-1.029)	**<.001***	1.015(1.005-1.025)	**0.004***
Left ventricular ejection fraction	0.936(0.908-0.965)	**<.001***	0.941(0.912-0.971)	**<.001***	0.951(0.921-0.982)	**0.002***
NT pro BNP	1(1-1)	0.155	1(1-1)	0.175	1(1-1)	0.086
White blood cell count	1(1-1)	0.941	1(1-1)	0.435	1(1-1)	0.081
History of Stroke	2.48(1.031-5.967)	**0.043***	2.636(1.092-6.362)	**0.031***	3.45(1.414-8.421)	**0.007***
Door-to-balloon time	1.002(0.989-1.015)	0.752	1.005(0.992-1.018)	0.445	1.005(0.993-1.018)	0.410
Infarct-related coronary artery						
Left anterior descending	1(reference)		1(reference)		1(reference)	
Left circumflex	2.344(0.895-6.139)	0.083	1.804(0.649-5.015)	0.258	1.463(0.493-4.34)	0.493
Right	2.576(0.983-6.746)	0.054	2.448(0.930-6.448)	**0.070**	2.079(0.774-5.58)	0.146
Left main	1.724(0.662-4.485)	0.264	1.693(0.662-4.332)	0.272	1.539(0.590-4.016)	0.379
DNI (at admission)	1.104(0.978-1.247)	0.110				
DNI (immediately after PCI)			1.065(1.031-1.101)	**<.001***		
DNI (24 hours after admission)					1.067(1.038-1.098)	**<.001***

(A)

	30-day mortality					
	Harrell's c-index	*p*-value	IDI	*p*-value	continuous NRI	*p*-value
Baseline (Cox model without DNI)	0.879 (0.814-0.932)					
Difference between Cox model						
with and without DNI at admission	0.004 (-0.024-0.005)	0.568	0.019 (-0.015-0.066)	0.306	0.147 (-0.295-0.54)	0.332
with and without DNI at immediately after PCI	0.009 (-0.028-0.003)	0.261	0.102 (0.103-0.181)	0.028	0.633 (0.118-0.776)	0.018
with and without DNI at 24hours after admission	0.013 (-0.035-0.002)	0.149	0.143 (0.013-0.231)	0.032	0.505 (-0.036-0.79)	0.076

(B)

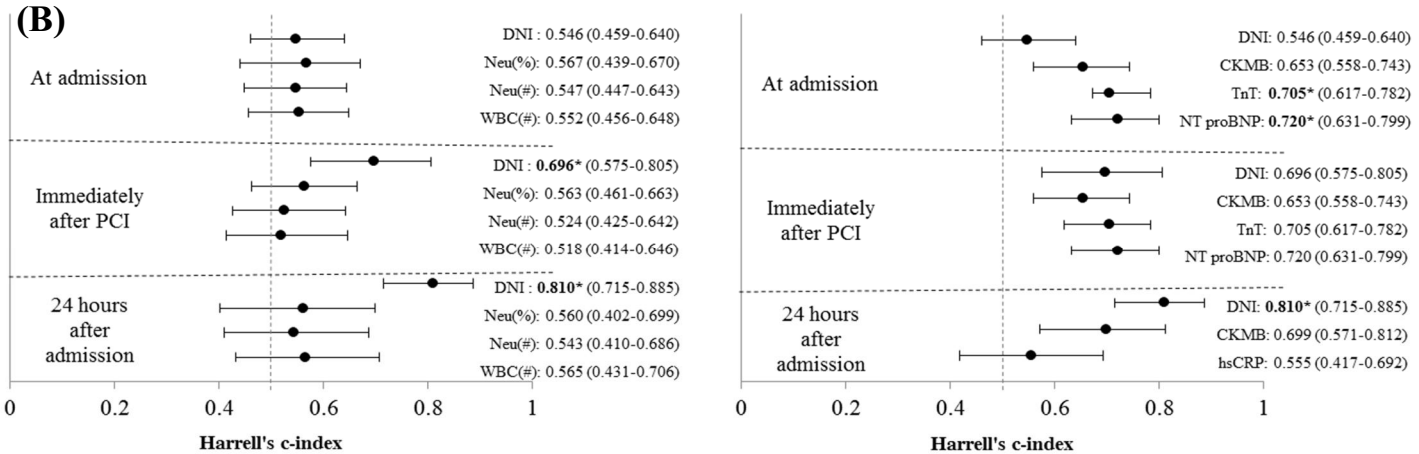

Figure 4. Discriminative ability of delta neutrophil index to predict mortality. (A) Comparison of the performance of the survival models with and without delta neutrophil index and (B) comparison of Harrell's c-index for biomarkers according to emergency department admission, immediately after reperfusion and 24 h after admission.

Limitations

This study has several limitations, including its retrospective design, and that the patient cohort was derived from a single, tertiary, academic hospital. Therefore, it was difficult to control for confounding factors, increasing the possibility of selection bias. However, we used the critical pathway that was prospectively performed with a standardized and predetermined protocol. Second, although our study demonstrated that the DNI during the early phase of STEMI was associated with 30-day mortality, we could not accurately assess the long-term clinical outcomes of patients with STEMI. Finally, despite using a prospective registry, serial measurements for indicators of severity of STEMI (such as CK-MB, Tn-T, hs-CRP and pro-inflammatory cytokines) are not mandatory in our FIRST protocol. Therefore, we were unable to serially evaluate all indicators of severity of STEMI at same time points at which values of DNI were measured. Further studies are required to validate and compare the usefulness of indicators of severity as prognostic markers in patients with STEMI.

V. CONCLUSION

DNI values, obtainable with no additional cost or time burden, can be measured rapidly and simply after ED admission. A high DNI level is an independent predictor of 30-day mortality in patients with acute STEMI post-pPCI.

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ABSTRACT (IN KOREAN)

일차 관상동맥 중재술을 시행한 ST분절 상승 심근경색 환자의 30일 사망 예측에 있어 델타 뉴트로필 지표의 유용성

<지도교수 박 인 철>

연세대학교 대학원 의학과

공 태 영

ST 분절 상승 심근경색증 (ST-segment elevation myocardial infarction, STEMI)은 치료법의 발전에도 불구하고 여전히 높은 사망률을 보이는 질환이다. 따라서 조기에 위험도를 정확히 평가하여 적극적이고 포괄적인 치료 전략을 적용하는 것이 중요하나, 이를 위한 뚜렷한 임상 지표는 없는 상태이다. 델타 뉴트로필 지표는 그동안 과도한 인체의 염증 반응을 수반하는 질환인 패혈증 및 심정지 환자에서의 예후 인자로서 유용성이 확인되었으며, 이는 허혈 재관류 시의 염증 반응이 예후에 결정적 역할을 하는 STEMI 환자에서도 유효할 것이라 가정할 수 있다. 이에 본 연구에서는 STEMI 환자에서의 30일 사망률 예측에 DNI가 유용한지 확인하고자 하였다.

본 연구는 2011년 1월부터 2016년 5월까지 연세대학교 세브란스병원 응급진료센터에 STEMI 로 내원하여 일차 경피적 관상동맥 중재술을 시행 받은 20세 이상의 성인 619명을 대상으로

하였으며, 후향적 의무기록 수집 및 통계분석을 실시하였다. 혼란변수를 통제한 다변량 분석 결과 델타 뉴트로필 지표가 일차 관상동맥 중재술 시행 직후 4.6% 이상, 내원 24시간째 4.8% 이상일 경우, 30일 내 사망을 독립적으로 예측할 수 있는 것으로 나타났다.

델타 뉴트로필 지표는 일반 혈액 검사에서 추가 비용없이 자동으로 측정이 가능한 매우 유용한 생체표지자이다. 델타 뉴트로필 지표의 증가가 STEMI 환자에서 30일 사망의 예측 인자로서 유용성이 확인되었으므로, 델타 뉴트로필 지표가 증가한 STEMI 환자의 경우 적극적 치료 전략의 적용 및 집중 모니터링이 필요할 것으로 생각된다.

핵심되는 말: ST 분절 상승 심근경색증, 델타 뉴트로필 지표, 사망률