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Diagnostic prediction model in subjects with low-risk unstable angina pectoris/Non-ST Segment Elevation Myocardial Infarction

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Abstract. – OBJECTIVE: This study aims to construct a prediction model based on non-invasive examination and cardiovascular risk factors, to predict the presence of coronary artery disease (CAD) and its severity in patients with low-risk unstable angina pectoris (UAP)/ Non-ST Segment Elevation Myocardial Infarction (NSTEMI).

PATIENTS AND METHODS: This cross-sectional study aimed to assess the association between non-invasive examinations and cardiovascular risk factors in predicting CAD. Model constructed based on non-invasive assessment and cardiovascular risk factors was compared to coronary angiography, the reference standard.

RESULTS: This study included 104 patients, comprising 60 men and 44 women, who fulfilled the inclusion criteria. The mean age was 52.3 (6.8) years. Two diagnostic prediction models were constructed after series of analyses. The main model consists of NO, CIMT, history of smoking, and Age-Gender, while the alternative model consists of CIMT, history of smoking, and Age-Gender. The main model has AUC of 74.5% (95% CI: 64.9-84.1), sensitivity of 72.7% (95% CI: 57.2-85.0), specificity 65.0% (95% CI: 51.6 -76.9 for a cut-off point of 74.5. While the alternative model has 69.0% AUC (95% CI: 58.9-79.1), sensitivity of 65.9% (95%: 50.1-79, 5), a specificity of 56.7% (95% CI: 43.2-69.4) for a cut-off point of 69. The main model and the alternative model have similar diagnostic prediction performance based on the ROC comparison test ($p = 0.70$).

CONCLUSIONS: Based on these results, we conclude that NO, CIMT, smoking history, and age-gender have a value of diagnostic validity in subjects with low-risk UAP/NSTEMI.

Key Words:
Acute coronary syndrome, Unstable angina pectoris, Coronary artery disease, Non-invasive, Diagnosis.

Introduction

Coronary artery disease (CAD) remains the leading cause of mortality and morbidity worldwide[1]. Early detection and diagnosis are crucial for reducing morbidity and mortality in patients with CAD. Early detection of CAD can be done with invasive and non-invasive methods. Non-invasive method is preferred because of its scalability, convenience, and cost-efficiency. Several non-invasive examinations are used to predict the presence of CAD, including Carotid Intima Media Thickness (CIMT), Ankle Brachial Index (ABI), Flow Mediated Dilatation (FMD), plasma levels of Endothelin-1 (ET-1), and Nitric Oxide (NO)[2-8]. However, which of these methods is the best remains controversial[5,6,9-11]. In addition to the non-invasive methods, some reports indicate that cardiovascular risk factors may also be used to predict CAD, although the evidence remains elusive[12-15].

Patients with acute coronary syndrome (ACS) present with acute chest discomfort due to atherosclerotic plaque rupture or erosion, causing thrombosis and impaired blood flow that can be life-threatening[16]. Patients with non-ST segment elevation acute coronary syndrome (NSTE-ACS) may present with or without signs of cardiomyocyte necrosis. Thus, NSTE-ACS was further classified into non-ST-segment elevation myocardial infarction (NSTEMI) and unstable angina pectoris (UAP) based on the presence of cardiomyocyte necrosis[16]. Patients with NSTE-ACS may demonstrate a wide range of symptoms from vague chest pain to hemodynamic instability and cardiac arrest. High-risk patients with hemodynamic instability and cardiac arrest usually underwent coronary an-

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giography[16]. However, the indication is often unclear in patients with low-risk UAP/NSTEMI. In low-middle income countries, coronary angiograms cannot be performed as generously as high-income countries due to economic and workforce limitations; thus, a non-invasive modality may be valuable. This study aims to construct a prediction model based on non-invasive examination and cardiovascular risk factors, to predict the presence of CAD and its severity in patients with low-risk UAP/NSTEMI. The presence of CAD and its severity was obtained through coronary angiography and the calculation of the Gensini score.

Patients and Methods

This cross-sectional study aimed to assess the association between non-invasive examination and cardiovascular risk factors in predicting coronary artery disease, proved by coronary angiography. This study was designed as a diagnostic test to assess the validity of a non-invasive and cardiovascular risk factor in predicting CAD based on coronary angiography findings. Model based on non-invasive assessment and cardiovascular risk factors was compared to coronary angiography, the reference standard. This study was conducted at the National Cardiovascular Center Harapan Kita (Jakarta, Indonesia). The target population in this study was all patients with UAP/NSTEMI with low TIMI risk stratification. The samples were obtained consecutively. The patients presented to the National Cardiovascular Center Harapan Kita with a diagnosis of UAP/NSTEMI with low TIMI risk stratification and underwent a coronary angiography examination. The dependent variable is CAD (as evidenced by coronary angiography). The independent variables consist of four aggregate variables, namely: history of illnesses (family history, smoking history, and age-gender), physical examination (obesity and hypertension), laboratory findings (dyslipidemia and diabetes mellitus), and the non-invasive vascular examinations, including Flow Mediated Dilation (FMD), Carotid Intima-Media Thickness (CIMT), Ankle Brachial Index (ABI), Endothelin-1 (ET-1) and Nitric Oxide /(NO).

All subjects who met the inclusion criteria following examination and coronary angiogram were divided into two groups, CAD and non-CAD group, based on coronary angiography findings. Criteria of CAD was based on coronary stenosis $\geq$ 50 % as proposed by ACC/AHA[17]. Laboratory tests ET-1 and NO and non-invasive vascular assessments (CIMT, ABI, FMD) were performed the following morning at the vascular clinic. ABI was performed manually using a pencil Doppler probe applied in both ankles and arms. The lowest measurement of both sides concluded as the ABI value of each subject. CIMT measurement method referred to the ASE recommendation and was performed using a semi-automated method called automatic border detection[18]. CIMT measurement was performed with GE Logic E ultrasound machine, equipped with a 11 Mhz Linear Probe (GE Healthcare Corp, Chicago, IL, USA). FMD was performed with the distal occlusion method proposed by Corretti et al[19], by using ALOKA Prosound α-100 ultrasound machine with 7.5 Mhz linear probe (Aloka UST-5710, Aloka Inc, Wallingford, CT, USA). A dedicated wall track system, called E-Tracking™ was applied to measure the FMD value automatically. A blood sample for endothelin-1 was drawn with 6 mL of blood collected in EDTA tube and then centrifuged at 300 rpm (1500 g) for 15 minutes. Plasma for ET-1 measurement then stored at minus 60°C. Plasma ET-1 was measured by ELISA method with Quantikinine Human Endothelin -1 Immunoassay Kit, catalog number DET-100 SET-100 R&D Systems (Inc., 614 McKinley Place NE, Minneapolis, MN 55413, USA)[20]. NO measurement was performed indirectly by measuring its metabolites (nitrate and nitrite) as proposed by Bryan et al[21]. Blood sample was drawn for 4 mL, then collected in a non-anticoagulant tube (plain tube), kept for 45-60 minutes until a clot formed, then centrifuged at 3000 RPM (1500 g). The serum was then collected and stored at 60°C until later used for measurement. Nitrate and nitrite measurement was performed with the Colorimetric technique; a Griess reaction method was applied to measure the sum of nitrite level, as nitrates will be changed to nitrites by nitrate reductase. This measurement was done using a Nitrate/Nitrite Colorimetric Assay kit catalog no 780001 from Cayman Chemical Co., (Ann Arbor, MI, USA)[22]. The results of coronary angiography were reviewed by one interventional cardiologist blinded to the patient condition to determine the degree of severity denoted by Gensini score. This study has been approved by the Research Ethics Committee of the Faculty of Medicine, University of Indonesia, and the Medical Research Ethics Committee of the National Cardiovascular Center Harapan Kita.

Statistical Analysis

Continuous data with normal distribution were presented as mean ± standard deviation (SD), and those without normal distribution were reported as median (minimum-maximum). Independent T-test was performed for normally distributed data and Mann-Whitney test for abnormally distributed data. Categorical variables were analyzed using Chi-square or Fischer exact test. Spearman correlation was performed for the CIMT and Gensini score. Risk ratios of the model and its associated variables were calculated using the linear regression test. The sensitivity, specificity, positive and negative likelihood ratios were then calculated for the optimal cut-off point derived from the receiver operating curve characteristics. All *p*-values in this study were two-tailed, and $p < 0.05$ indicates statistical significance. The data was analyzed using SPSS 25.0 (IBM Corp, Armonk, NY, USA).

Results

During 15 months, there were 1827 patients with UAP/NSTEMI, and 124 of them were stratified as low-risk UAP/NSTEMI. This study included 104 patients, comprising 60 men and 44 women, who fulfilled the inclusion criteria. The mean age was 52.3 (6.8) years. The majority of subjects (60.6%) was overweight. Approximately 38.5% of the study subjects have a history of smoking. Baseline characteristics of the included subjects are shown in Table I.

There were 44 patients with CAD and 60 patients with normal coronary arteries. The median and range of non-invasive measurements based on the presence of CAD are shown in Table II. Among the non-invasive measurements, NO demonstrated the strongest association with the presence of CAD (RR 1.97; 95% CI 1.17 - 3.31; $p = 0.006$). NO above 7.3 has a sensitivity of 70 % for the presence of CAD, as shown in Table III. The association between non-invasive measurement and CAD is shown in Table IV. The association of risk factors and non-invasive measurements with the diagnostic prediction model is shown in Table V. This prediction model was taken by multivariate analysis with Linear Regression analysis. Two diagnostic prediction models were constructed, the main model consists of NO, CIMT, history of smoking, and Age-Gender, while the alternative model consists of CIMT, history of smoking, and Age-Gender. The main model has AUC of 74.5% (95% CI: 64.9-84.1), sensitivity of 72.7% (95% CI: 57.2-85.0), specificity 65.0% (95% CI: 51.6 -76.9 for a cut-off point of 74.5. While the alternative model has 69.0% AUC (95% CI: 58.9-79.1), sensitivity of 65.9% (95%: 50.1-79, 5), a specificity of 56.7% (95% CI: 43.2-69.4) for a cut-off point

Table I. Subject characteristics.

		Mean (SE)	N	%
Age (years)*		52.3 (6.8)		
Gender	Men		60	57.7
	Women		44	42.3
BMI Classification (Asia Pacific)	Underweight (< 18.5)		1	1.0
	Normal (18.5-22.9)		20	19.2
	At risk of obesity (23.0-24.9)		22	21.2
	Obesity I (25-29.9)		50	48.1
	Obesity II (≥ 30.0)		11	10.6
Diabetes	Yes		26	25.0
	No		78	75.0
Cigarette Smoking	Yes		40	38.5
	No		64	61.5
Dyslipidemia	Yes		66	63.5
	No		38	36.5
Hypertension	Yes		47	45.2
	No		57	54.8
Total TIMI score	0/7		2	1.9
	1/7		38	36.5
	2/7		64	61.5
Diagnosis	UAP		98	94.2
	NSTEMI		6	5.8
Total			104	100

Table II. Median and range of non-invasive measurements based on CAD.

Variable	CAD median (Min-Max)	Non CAD median (Min-Max)	p#
CIMT (mm)	0.92 (0.60-2.50)	0.84 (0.54-1.72)	0.020
FMD (%)	6.35 (.98-32.94)	8.18 (0.57-35.90)	0.242
ABI	1.08 (0.75-1.30)	1.07 (0.89-1.30)	0.665
NO (pg/mL)	6.75 (2.80-26.70)	7.55 (2.00-44.20)	0.152
ET-1(µM/L)	1.27 (0.12-4.04)	1.23 (0.07-6.06)	0.541
Ratio NO/ET-1	5.49 (0.79-95.83)	6.42 (1.39-76.00)	0.186

#Mann-Whitney test.

Table III. Diagnostic prediction value of non-invasive measurement.

	AUC	Cut off	Sensitivity (%)	Specificity (%)
ET-1 (pg/mL)	53.5 (42.3-64.7)	1.005*	70.5	35.0
NO (µM/L)	58.2 (47.1-69.4)	7.3**	70.5	66.7
CIMT (mm)	63.4 (52.2-74.6)	0.8175*	72.7	45.0
FMD (%)	56.7 (45.6-67.9)	11.615**	70.5	36.7
ABI	52.1 (40.8-63.4)	1.035**	70.5	33.3
Ratio NO/ET-1	57.6 (46.5-68.8)	6.71**	70.5	48.3

*Indicator of CAD if the value ≥ cut off point. **Indicator of CAD if the value ≤ cut off point.

of 69. The main model and the alternative model have similar diagnostic prediction performance based on the ROC comparison test (p = 0.70) (Table VI).

Correlation Between CIMT and CAD Severity

There was a weak positive correlation between the CIMT and Gensini score (r = 0.22 and p = 0.006) (Figure 1 and Figure 2).

Discussion

CIMT and CAD

The cut-off point for CIMT in our study was 0.82 mm, which means that CIMT ≥ 0.82 mm is classified as abnormal. This cut-off point is similar to the existing literature in the range of 0.65-1.0 mm[5,6,10,23-25]. There was a significant difference in terms of CIMT between CAD (0.92 mm) and non-CAD groups (0.84 mm), which is

Table IV. Association between non-invasive measurements and CAD.

		CAD		Non CAD			Adjusted	95% CI	
	Cut off*	N	%	N	%	p	RR	Min	MAx
CIMT (mm)	≥ 0.82	32	49.2	33	50.8	0.065	1.60	0.94	2.72
	< 0.82	12	30.8	27	69.2		Ref		
NO (µM/L)	≤ 7.30	31	54.4	26	45.6	0.006	1.97	1.17	3.31
	> 7.30	13	27.7	34	72.3		Ref		
FMD (%)	≤ 11.62	31	44.9	38	55.1	0.448	1.21	0.73	2.00
	> 11.62	13	37.1	22	62.9		Ref		
ET-1 (pg/mL)	≥ 1.01	31	44.3	39	55.7	0.558	1.16	0.70	1.91
	< 1.01	13	38.2	21	61.8		Ref		
ABI	≥ 1.04	31	43.7	40	56.3	0.682	1.11	0.67	1.83
	< 1.04	13	39.4	20	60.6		Ref		
Rasio NO/ET-1	≤ 6.71	31	50.0	31	50.0	0.054	1.62	0.96	2.71
	> 6.71	13	31.0	29	69.0		Ref		
Total		44	42.3	60	57.7				

*Cut off based on sensitivity of 70%.

Table V. Association of risk factors and non-invasive measurements with diagnostic prediction model.

	B	SE	Wald	Df	p	Adjusted RR	95% CI	
							Min	Max
Age-Gender	0.518	0.373	1.931	1	0.165	1.68	0.81	3.49
Cigarette smoking (+)	0.422	0.314	1.803	1	0.179	1.53	0.82	2.82
CIMT ≥ 0.82 mm	0.308	0.349	0.779	1	0.377	1.36	0.69	2.70
NO ≤ 7.30 μM/L	0.674	0.331	4.129	1	0.042	1.96	1.02	3.76

consistent with previous reports by Teragawa et al[5], Furumoto et al[6] and Kaku et al[9], which found that CIMT was closely associated with CAD. Holland et al[26] concluded that the increase in CIMT is associated with future cardiovascular and stroke events. Cicorella et al[2], Heuten et al[27] and Kasliwal et al[28] described that CIMT was correlated with the left main disease.

NO and CAD

The level of NO was represented by its metabolites (nitrite and nitrates) in this study. The concentration of NO was lower in patients with CAD compared to non-CAD. The cut-off point was determined to be 7.3 μM/L. This cut-off point was lower than previously reported by Segzin et al[29], Ferlito et al[30] and Akiyama et al[31].

Akiyama et al[31] used chemiluminescent methods, which are different to spectrophotometry; however, whether this difference affects the result remains to be investigated.

On the other hand, de Miguel et al[32] showed that plasma NO in patients with UAP was relatively lower than patients with STEMI because the neutrophil of STEMI patients produces more NO compared to UAP patients.

Another factor to be considered in Akiyama et al[31] study was that patients undergoing coronary angioplasty have significantly increased NO levels. Although there was no reliable data on the effect of coronary angioplasty on NO, a study reported that coronary angiography increases the level of NO[33].

Upchurch et al[34] reported that a decreased NO in patients with ACS is related to heparin. Heparin is routinely given in ACS will lead to inhibition of cGMP production by platelets. Another possible explanation is the decreased level of steady-state NOS III mRNA and destabilization of extracellular NO due to the release of Super Oxide Dismutase from the cell surface[34].

Another explanation of the reduced amount of NO level during ACS is related to endothelin-1, which leads to NO production due to the inhibitory mechanism of eNOS expression from endothelial cells by a specific isoform of PKC as proposed by Ramzy et al[35] and Freedman et al[36] demonstrated that reducing of NO production is related to platelet aggregation and thrombus formation in acute coronary syndrome. Bioavailability of NO is determined by many causes, including oxidative stress[8]. Thus, our finding regarding a relatively lower level of NO in CAD subjects is consistent with the previous report as mentioned above. A rapid NO examination kit, which measures nitrate and nitrite similar to that of the rapid troponin kit, is needed to improve the scalability of the main model.

Table VI. Comparison of 2 diagnostic prediction models.

	Main Model	Alternative Model	p
AUC; % (95% CI)	y = -0.146xtotal score 1	y = -0.154xtotal score 2	0.170*
Score Cut off sk	74.5 (64.9-84.1)	69.0 (58.9-79.1)	
Sensitivity; % (95% CI)	8	5	
Specificity; % (95% CI)	72.7% (57.2-85.0)	65.9% (50.1-79.5)	
PPV;% (95% CI)	65% (51.6-76.9)	56.7% (43.2-69.4)	
NPV;% (95% CI)	60.4% (46.0-73.5)	52.7% (38.8-66.3)	
Likelihood Ratio (+) (95% CI)	76.5% (62.5-87.2)	69.4% (54.6-81.7)	
Likelihood Ratio (-) (95% CI)	2.08 (1.41-3.07)	1.52 (1.06-2.18)	
	0.42 (0.25-0.70)	0.60 (0.38-0.96)	

*ROC comparison test.

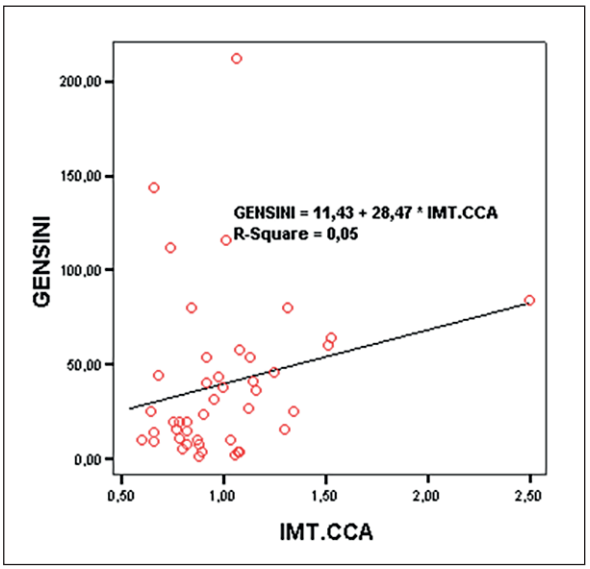

Figure 1. Correlation between CIMT and CAD severity.

Application of the Scoring System

The widely used scoring systems such as Framingham Risk Score, TIMI, GRACE, and PURSUIT emphasize prognostic value[24,37,38]. These scores were developed to predict major adverse cardiovascular events, additionally, most of them are not specific to ACS. Duke Score and Diamond Forrester Score are the scoring systems with diagnostic purpose; however, they are not specific for low-risk ACS[39,40]. To the best of the authors' knowledge, there was no broadly accepted scoring system for low-risk UAP/NSTEMI. Therefore, our study aimed to fulfill the gap.

Although these scoring systems were not designed to replace coronary CT scans for the screening of low-risk UAP/NSTEMI patients[40-42], they can help make a decision in triage units or hospitals without CT scan facilities.

An alternative model was constructed to meet the demands of regional or district hospitals throughout the country, in which NO measurement may not be possible. Nevertheless, CIMT assessment is operator-dependent, and emergency physicians in the respective hospitals must be trained to operate the ultrasound properly.

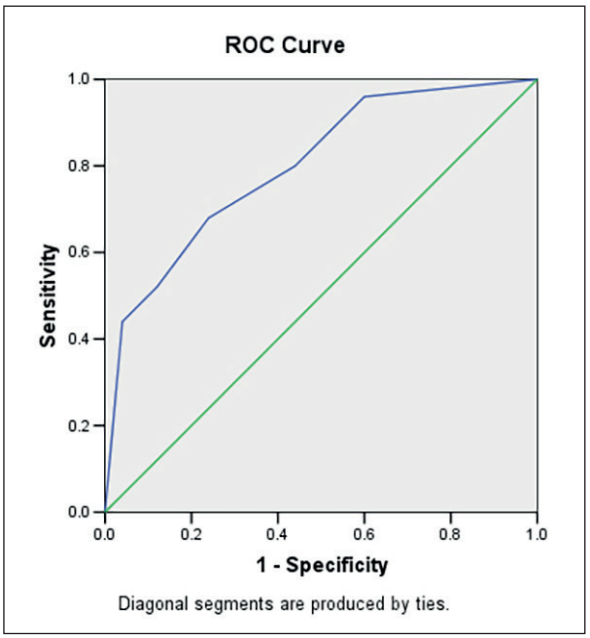

Figure 2. ROC Curve of Validation Alternative model.

ASPECT Study and Recommendations

Asia Pacific Evaluation of Chest Pain (ASPECT) Trial is a study designed to validate the Accelerated Diagnostic Protocol (ADP), which may help determine candidates for early discharge. This ADP is aimed to shorten observation time in the emergency unit, which may alleviate the economic burden[41].

One of the primary weaknesses of the ASPECT study is that, even if the ADP allows early discharge of patients, we cannot rule out the potential for other fatal conditions with absolute certainty. That is, although the protocol is designed to rule out ACS, other life-threatening conditions such as aortic dissection, cardiac tamponade, tension pneumothorax, pulmonary emboli, etc., might be missed. This may lead to medico-legal issues[42]. Additionally, ASPECT study was based on prognosis, such as major adverse cardiovascular events, rather than diagnosis[41]. The result of our study may help in ruling out patients without CAD.

Conclusions

Based on these results, we conclude that NO, CIMT, smoking history and age-gender has a value of diagnostic validity in subjects with low-risk UAP/NSTEMI. Model that consists of CIMT, NO, History of smoking, and Age-gender has 74.5% of AUC, sensitivity of 72.7% and specificity of 65% for predicting the presence of CAD. Additionally, there was a weak positive correlation between CIMT with CAD severity. In conclusion, this study provides a reliable diagnostic prediction model for the presence of CAD based on parameters that can be obtained through history taking and non-invasive assessment in patients with low-risk UAP/NSTEMI

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Tyroler HA. Coronary heart disease epidemiology in the 21st century. Epidemiol Rev 2000; 22: 7-13.
2) Cicorella N, Zanolla L, Franceschini L, Cacici G, De Cristan B, Arieti M, Vassanelli C. Usefulness of ultrasonographic markers of carotid atherosclerosis (intima-media thickness, unstable carotid plaques and severe carotid stenosis) for predicting presence and extent of coronary artery disease. J Cardiovasc Med 2009; 10: 906-912.
3) Weatherley BD, Nelson JJ, Heiss G, Chambless LE, Richey AR, Javier FJ, Folsom AR, Rosamond WD. The association of the ankle-brachial index with incident coronary heart disease: the Atherosclerosis Risk In Communities (ARIC) study, 1987-2001. BMC Cardiovasc Disord 2007; 7: 3.
4) Núñez D, Morillas P, Quiles J, Cordero A, Guindo J, Soria F, Mazón P, Lekuona I, Rodríguez-Padial L, Llácer Á, González-Juanatey JR, Bertomeu V. Usefulness of an abnormal ankle-brachial index for detecting multivessel coronary disease in patients with acute coronary syndrome. Rev Española Cardiol 2010; 63: 54-59.
5) Teragawa H, Kato M, Kurokawa J, Yamagata T, Matsuura H, Chayama K. Usefulness of flow-mediated dilation of the brachial artery and/or the intima-media thickness of the carotid artery in predicting coronary narrowing in patients suspected of having coronary artery disease. Am J Cardiol 2001; 88: 1147-1151.
6) Furumoto T, Fujii S, Saito N, Mikami T, Kitabatake A. Relationships between brachial artery flow mediated dilation and carotid artery intima-media thickness in patients with suspected coronary artery disease. Jpn Heart J 2002; 43: 117-125.
7) Zouridakis EG, Schwartzman R, Garcia-Moll X, Cox ID, Fredericks S, Holt DW, Kaski JC. Increased plasma endothelin levels in angina patients with rapid coronary artery disease progression. Eur Heart J 2001; 22: 1578-1584.
8) Higashino H, Tabuchi M, Yamagata S, Kurita T, Miya H, Mukai H, Miya Y. Serum nitric oxide metabolite levels in groups of patients with various diseases in comparison of healthy control subjects. J Med Sci 2010; 10: 1-11.
9) Kaku B, Mizuno S, Ohsato K, Murakami T, Moriuchi I, Aral Y, Nio Y, Hirase H, Nagata M, Takahashi Y, Ohnaka M. The correlation between coronary stenosis index and flow-mediated dilation of the brachial artery. Jpn Circ J 1998; 62: 425-430.
10) Bampi ABA, Rochitte CE, Favarato D, Lemos PA, da Luz PL. Comparison of non-invasive methods for the detection of coronary atherosclerosis. Clinics 2009; 64: 675-682.
11) Gullu H, Erdogan D, Caliskan M, Tok D, Yildirim E, Ulus T, Turan Sezgin A, Muderrisoglu H. Interrelationship between noninvasive predictors of atherosclerosis: transthoracic coronary flow reserve, flow-mediated dilation, carotid intima-media thickness, aortic stiffness, aortic distensibility, elastic modulus, and brachial artery diameter. Echocardiography 2006; 23: 835-842.
12) Liu J, Hong Y, D'Agostino RB, Wu Z, Wang W, Sun J, Wilson PWF, Kannel WB, Zhao D. Predictive value for the Chinese population of the Framingham CHD risk assessment tool compared with the Chinese Multi-provincial Cohort Study. J Am Med Assoc 2004; 291: 2591-2599.
13) Rivera JJ, Nasir K, Cox PR, Choi EK, Yoon Y, Cho I, Chun EJ, Choi S Il, Blumenthal RS, Chang HJ. Association of traditional cardiovascular risk factors with coronary plaque sub-types assessed by 64-slice computed tomography angiography in a large cohort of asymptomatic subjects. Atherosclerosis 2009; 206: 451-457.
14) Bosch X, Monclús E, Escoda O, Guerra-García M, Moreno P, Guasch N, López-Soto A. Unintentional weight loss: clinical characteristics and outcomes in a prospective cohort of 2677 patients. PLoS One 2017; 12: e0175125.
15) Han JH, Lindsell CJ, Storrow AB, Luber S, Hoekstra JW, Hollander JE, Peacock IV WF, Pollack CV, Gibler WB. The role of cardiac risk factor burden in diagnosing acute coronary syndromes in the emergency department setting. Ann Emerg Med 2007; 49: 145-152.
16) Collet JP, Thiele H, Barbato E, Bauersachs J, Dendale P, Edvardsen T, Gale CP, Jobs A, Lambrinou E, Mehilli J, Merkely B, Roffi M, Sibbing D, Kastrati A, Mamas MA, Aboyans V, Angiolillo DJ, Bueno H, Bugiardini R, Byrne RA, Castelletti S, Chieffo A, Cornelissen V, Crea F, Delgado V, Drexel H, Gierlotka M, Halvorsen S, Haugaa KH, Jankowska EA, Katus HA, Kinnaird T, Kluin J, Kunadian V, Landmesser U, Leclercq C, Lettino M, Meinila L, Mylotte D, Ndrepepa G, Omerovic E, Pedretti RFE, Petersen SE, Petronio AS, Pontone G, Popescu BA, Potpara T, Ray KK, Luciano F, Richter DJ, Shlyakhto E, Simpson IA, Sousa-Uva M, Storey RF, Touyz RM, Valgimigli M, Vranckx P, Yeh RW, Barthélémy O, Bhatt DL, Dorobantu M, Folliguet T, Gilard M, Jüni P, Lewis BS, Meliga E, Mueller C, Rutten FH, Siontis GCM. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J 2021; 42: 1289-1367.
17) Scanlon PJ, Faxon DP, Audet AM, Carabello B, Dehmer GJ, Eagle KA, Legako RD, Leon DF, Murray JA, Nissen SE, Pepine CJ, Watson RM, Ritchie JL, Gibbons RJ, Cheitlin MD, Gardner TJ, Garson A, Russell RO, Ryan TJ, Smith SC. ACC/AHA Guidelines for Coronary Angiography. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Coronary Angiography). J Am Coll Cardiol 1999; 33: 1756-1824.
18) Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post

WS. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008; 21: 93-111.

19) Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the international brachial artery reactivity task force. J Am Coll Cardiol 2002; 39: 257-265.
20) Quantikine Human Endothelin-1 Quantikine ELISA,Catalog Number DET-100 SET-100 R&D Systems Inc.
21) Bryan NS, Grisham MB. Methods to detect nitric oxide and its metabolites in biological samples. Free Radic Biol Med 2007; 43: 645-657.
22) Nitrate/Nitrite Colorimetric Assay Kit Catalog No. 780001.
23) Mutlu B, Tigen K, Gurel E, Ozben B, Karaahmet T, Basaran Y. The predictive value of flow-mediated dilation and carotid artery intima-media thickness for occult coronary artery disease. Echocardiography 2011; 28: 1141-1147.
24) Wilson PWF, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998; 97: 1837-1847.
25) D'Agostino RB, Russell MW, Huse DM, Ellison RC, Silbershatz HC, Wilson PWF, Hartz SC. Primary and subsequent coronary risk appraisal: new results from the Framingham study. Am Heart J 2000; 139: 272-281.
26) Holland Z, Ntyintyane LM, Gill G V., Raal FJ. Carotid intima-media thickness is a predictor of coronary artery disease in South African black patients. Cardiovasc J Afr 2009; 20: 237-239.
27) Heuten H, Goovaerts I, Ennekens G, Vrints C. Carotid artery intima-media thickness is associated with coronary artery disease. Acta Cardiol 2008; 63: 309-313.
28) Kasliwal RR, Bansal M, Gupta H, Agrawal S. Association of carotid intima-media thickness with left main coronary artery disease. Indian Heart J 2007; 59: 50-55.
29) Sezgin N, Barutcu I, Sezgin TA, Gullu H, Turkmen M, Esen AM, Karakaya O. Plasma nitric oxide level and its role in slow coronary flow phenomenon. Int Heart J 2005; 46: 373-382.
30) Ferlito S, Gallina M. Nitrite plasma levels in type 1 and 2 diabetics with and without complications. Minerva Endocrinol 1999; 24: 117-121.
31) Akiyama K, Kimura A, Suzuki H, Takeyama Y, Gluckman TL, Terhakopian A, Katagiri T, Suh KY, Roseto J, Bing RJ. Production of oxidative products of nitric oxide in infarcted human heart. J Am Coll Cardiol 1998; 32: 373-379.
32) Sánchez de Miguel L, Arriero M a. M, Farré J, Jiménez P, García-Méndez A, De Frutos T, Jiménez A, García R, Cabestrero F, Gómez J, De Andrés R, Montón M, Martín E, De la Calle-Lombana LM, Rico L, Romero J, López-Farré A. Nitric oxide production by neutrophils obtained from patients during acute coronary syndromes: expression of the nitric oxide synthase isoforms. J Am Coll Cardiol 2002; 39: 818-825.
33) Domaniç N, Gelişgen R, Civelek S, Demir AS, Ural D, Andican G, Vural VA, Burçak G. Homocysteine and nitric oxide in patients undergoing diagnostic coronary angiography. Acta Med Okayama 2006; 60: 35-41.
34) Upchurch GR, Welch GN, Freedman JE, Fabian AJ, Pigazzi A, Scribner AM, Alpert CS, Keaney JF, Loscalzo J. High-dose heparin decreases nitric oxide production by cultured bovine endothelial cells. Circulation 1997; 95: 2115-2121.
35) Ramzy D, Rao V, Tumiati LC, Xu N, Sheshgiri R, Miriuka S, Delgado DH, Ross HJ. Elevated endothelin-1 levels impair nitric oxide homeostasis through a PKC-dependent pathway. Circulation 2006; 114: I319-I326.
36) Freedman JE, Ting B, Hankin B, Loscalzo J, Keaney JF, Vita JA. Impaired platelet production of nitric oxide predicts presence of acute coronary syndromes. Circulation 1998; 98: 1481-1486.
37) Antman EM, Cohen M, Bernink PJLM, McCabe CH, Horacek T, Papuchis G, Mautner B, Corbalan R, Radley D, Braunwald E. The TIMI risk score for unstable angina/non-ST elevation MI: a method for prognostication and therapeutic decision making. J Am Med Assoc 2000; 284: 835-842.
38) De Araújo Gonçalves P, Ferreira J, Aguiar C, Seabra-Gomes R. TIMI, PURSUIT, and GRACE risk scores: Sustained prognostic value and interaction with revascularization in NSTE-ACS. Eur Heart J 2005; 26: 865-872.
39) Kwok JMF, Miller TD, Hodge DO, Gibbons RJ. Prognostic value of the Duke treadmill score in the elderly. J Am Coll Cardiol 2002; 39: 1475-1481.
40) Diamond GA, Forrester JS. Analysis of probability as an aid in the clinical diagnosis of coronary-artery disease. N Engl J Med 1979; 300: 1350-1358.
41) Than M, Cullen L, Reid CM, Lim SH, Aldous S, Ardagh MW, Peacock WF, Parsonage WA, Ho HF, Ko HF, Kasliwal RR, Bansal M, Soerianata S, Hu D, Ding R, Hua Q, Seok-Min K, Sritara P, Sae-Lee R, Chiu TF, Tsai KC, Chu FY, Chen WK, Chang WH, Flaws DF, George PM, Richards AM. A 2-h diagnostic protocol to assess patients with chest pain symptoms in the Asia-Pacific region (ASPECT): a prospective observational validation study. Lancet 2011; 377: 1077-1084.
42) Jones ID, Slovis CM. Pitfalls in evaluating the low-risk chest pain patient. Emerg Med Clin North Am 2010; 28: 183-201.