The Cut-off Point of Interleukin-6 Level in Acute Coronary Syndrome

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ABSTRACT

Aim: this study aimed to observe whether the interleukin-6 level in acute coronary syndrome (ACS) patients were higher than those in coronary heart disease (CHD) patients. In addition, we would like to observe the cut off point of interleukin-6 level in ACS.

Methods: this cross sectional study were conducted at Dr. Cipto Mangunkusumo General Hospital (RSUPN-CM), Persahabatan Hospital, MMC Hospital and Medistra Hospital, Jakarta. The study started from 1 May 2005 to 5 May 2006.

Results: in this observational study, as many as 62 CHD patients were collected and 84 ACS that met the study criteria. Demographic analysis showed that there was no difference in ages among the two groups (ACS and CHD). The risk factors of dyslipidemia, hypertension and lipid profile in the two groups did not differ significantly. Waist circumference and IMT, systolic and diastolic blood pressures in the two groups did not also differ significantly. Smoking was more prevalent in the groups of ACS than in the groups of CHD. In this study the IL-6 level in ACS (mean 40.85pg/mL, SD 41.71, CI 95% 25.63-42.08) was higher than that in CHD (mean 4.58 pg/mL, SD 9.61, CI 95% 2.14-7.02). To identify the IL-6 level as the predictor for the occurrence of ACS, sensitivity and specificity were calculated at various cut-off points of IL-6 level. At cut-off point of IL-6 ≥ 4.43 pg/mL the highest sensitivity (89.95%) and highest specificity (77.42%) were found with ROC of 0.87.

Conclusion: it could be concluded that the IL-6 level in ACS were higher that those in CHD. The IL-6 level ≥ 4.43 pg/mL could differentiate the acute condition (ACS) and stable condition (non-ACS) with sensitivity of 89.95% and specificity of 77.42%, and ROC of 0.87.

Key words: interleukin-6, acute coronary syndrome.

INTRODUCTION

Atherosclerosis and its associated manifestations including acute coronary syndrome (ACS), has been proven as a chronic inflammatory disease.1-4 Acute coronary syndrome is associated with release of proinflammatory mediators from macrophage and T-lymphocytes, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6). These cytokines will stimulate production of acute phase reactants, C-reactive protein (CRP), in the liver.5 Besides CRP, other inflammatory marker commonly being studied is proinflammatory cytokines.5,7 Studies revealed that the level of proinflammatory cytokines such as IL-1β and IL-6 increase in stable8 and unstable angina pectoris,9 and also in acute myocardial infarction (AMI).10 Serum interleukin-6 level is a risk factor for AMI in healthy men.11

Studies on serum interleukin-6 level in ACS, especially to establish an interleukin-6 cutoff level in ACS patients in Indonesia, have never been reported.

METHODS

This is a comparative observational cross-sectional study. Patients in ACS group is compared with patients in CHD group. Measurement of serum IL-6 level could well illustrate proinflammatory response, both acute and chronic.

Time and Location of Study

The study was performed at the intensive coronary care unit (ICCU) in Cipto Mangunkusumo National General Hospital (CMNGH)/Faculty of Medicine University of Indonesia (FMUI), Persahabatan Hospital, Metropolitan Medical Center (MMC) Hospital, Medistra Hospital, Cardiology Outpatient Clinic Department of Internal Medicine FMUI-CMNGH, and Integrated Heart Service Outpatient Clinic CMNGH. The study was performed from May 2005 to May 2006.
Patient Population

Subjects being studied were ACS patients being hospitalized at the ICCU of CMNGH, Persahabatan Hospital, MMC Hospital, and Medistra Hospital. Patients were matched for age and sex. Control group for CHD was taken from patients visiting Cardiology Outpatient Clinic Department of Internal Medicine FMUI-CMNGH and Integrated Heart Service Outpatient Clinic CMNGH.

Informed consent will be obtained from the patient after the patient is given explanations from the researcher / research assistant. Permission to perform the study was obtained from FMUI Research Ethics Committee.

Estimation of Sample Size

In this part the inflammatory response values in ACS was compared with that in CHD. Estimation of sample size used the t-test formula for 2 independent groups: \( n = \frac{2(\sigma^2 + z^2\beta^2)}{d^2} \); with \( n \): number of subjects in each group, \( \sigma \): standard deviation of dependent variable, \( Z\sigma \): normal standard deviation for \( \alpha \); if \( \alpha = 0.05 \), two-way test, then \( Z\sigma = 1.96 \); \( Z\beta \): normal standard deviation for \( \beta \); if \( \beta = 0.20 \), then \( Z\beta = 0.84 \); \( d \): mean difference of ACS patients compared to control group.

Inflammatory Response

From previous studies we found some inflammatory response parameters as follows: mean serum IL-6 value in a study by Smith et al.\(^{12}\) in unstable angina pectoris (UAP) 20.9 pg/mL (SD=18.7) and in stable angina pectoris (SAP) 11.4 pg/mL (SD=4.2).

Inclusion and Exclusion Criteria

The inclusion criteria were ACS patients undergoing hospitalization in ICCU CMNGH, Persahabatan Hospital, MMC Hospital, and Medistra Hospital fulfilling ACS criteria with onset <72 hours, CHD patients visiting CMNGH Cardiology Outpatient Clinic and Integrated Heart Service Outpatient Clinic, who are willing to participate in the study and sign the informed consent.

The exclusion criteria were currently having acute myocardial infarction, smoking habit, previous history of medication, history of hypertension, history of diabetes mellitus, history of dyslipidemia, family history of heart disease. Physical examination: blood pressure, heart physical examination, body height, body weight, waist circumference.

Clinical laboratory examination included peripheral blood count: hemoglobin, hematocrit, leukocyte, thrombocyte; CK, CKMB, troponin T, fasting and postprandial serum glucose concentration, glyco Hb, total cholesterol level, direct LDL cholesterol level, HDL cholesterol level and triglyceride, ureum, creatinine, SGOT, SGPT. Inflammatory response: serum IL-6 level.

Acute coronary syndrome (ACS)\(^{13,14}\): a spectrum of heart emergency consists of: acute myocardial infarction with ST elevation (ST-elevated myocardial infarction/STEMI), acute myocardial infarction without ST elevation (non ST-elevated myocardial infarction/NSTEMI), unstable angina pectoris (UAP).

Unstable angina pectoris\(^{15}\) was defined according to Braunwald’s classification (fulfilling one of the following criteria): 1. Angina found during rest and lasts for a long time, usually lasts more than 20 minutes. 2. New onset angina classified as IIIrd class or above in the Canadian Cardiovascular Society (CCS) classification. 3. Acceleration of new angina with signs of increased severity of angina at least 1 CCS to at least IIIrd class of CCS.

Canadian Classification of Angina;\(^{16}\) I. Angina only occurs with severe activity. II. Moderate activity, such as walking more than 1 floor, causes angina. III. Mild activity, such as walking less than 1 floor, causes angina. IV. All activities, even resting, causes angina.

Criteria used to diagnose acute myocardial infarction (AMI)\(^{17}\) were characteristic elevation and gradual decline (troponin) or earlier elevation and decline of biochemical markers of myocardial necrosis with at least one of the following symptoms: a. Ischemic symptoms, b. Occurrence of pathologic Q wave in the ECG. c. Changes in ECG waves indicating ischemia (elevation or depression of ST segment). d. Coronary artery intervention (e.g. coronary angioplasty).

Coronary heart disease (CHD) was defined as a coronary heart disease patients not currently having acute coronary syndrome based on anamnesis and ECG, and proved having a history of CHD based on previous coronary angiography examination or having a history of acute myocardial infarction/ACS at least 6 months before the examination.
Age was measured as the age on the day of examination according to the Citizen Identification Card, if over 6 months should be rounded up and if below 6 months should be rounded down. Body weight: in kilogram (kg) measured using SECA digital 770 weighing scale. Body height: in centimeter (cm) measured using Microtois CMS equipment. Body mass index (BMI): in kg/m$^2$ calculated using the following formula: body weight (kg) / body height (m)$^2$. Waist circumference is measured using SECA 200 measuring tape.

**Inflammatory Response**

Serum interleukin-6 level: in pg/mL, using enzyme immunoassay method. Reagent being used is Quantikine$^\text{R}$ from R&D System, Inc. 614 McKinley Place NE, Minneapolis, MN 55413, USA. Normal value ranges between 0.447 - 9.96 pg/mL. Intra assay variability is 3.8 - 11.1 % and inter assay variability is 9.9 - 16.5%.

**Laboratory Assays**

For routine blood count and blood chemistry analysis, fasting peripheral venous blood sample (10-12 hours) was collected, then the second blood sample was taken for 2 hour postprandial blood glucose level, using standard method, and sent to the Clinical Laboratory. Sample for serum IL-6 level measurement was taken from venous blood using standard method and sent to the Clinical Laboratory.

ACS patients being hospitalized at the ICCU of CMNGH, Persahabatan Hospital, MMC Hospital, and Medistra Hospital were selected using consecutive sampling between May 2005 and May 2006, while CHD patients were selected using consecutive sampling at the CMNGH Cardiology Outpatient Clinic and Integrated Heart Service Outpatient Clinic within the same period of time. CHD patients were only taken from CMNGH Outpatient Clinic because the number of cases was enough so that the samples did not have to be collected from other hospitals.

**Statistical Analysis**

All data obtained were continuous data. The data were coded, tabulated using STATA, and calculated statistically. Normally distributed variables went through parametric tests, and variables with abnormal distribution went through transformation, so as to be compliant with parametric test. All calculations used $p < 0.05$ as the level of significance, calculated using STATA statistical program.

In order to compare inflammatory response between ACS and CHD patients, serum IL-6 level variable, continuous, abnormal distribution, logarithmic transformation was performed in order to obtain a normal data distribution, parametric statistical test was used; the t test for different samples.

**RESULTS**

**Serum IL-6 Level in ACS Group and CHD Group**

In this study, the serum IL-6 level in ACS (mean 40.85 pg/mL, SD 51.71, CI 95 % 29.63-52.08) is higher compared to CHD (mean 4.58 pg/mL, SD 9.61, CI 95 % 2.14-7.02). Table 1 demonstrates serum IL-6 level at cut-off point $\geq$ 4.43 pg/mL shows highest sensitivity and specificity.

**Cutoff Point of Serum IL-6 Level in ACS Group Compared to CHD Group**

In order to investigate serum IL-6 level as a predictor of ACS, sensitivity and specificity of serum IL-6 level were calculated for the ACS group in various levels of serum IL-6. In serum IL-6 cutoff point $\geq$ 4.43 pg/mL, we obtained the highest sensitivity and specificity.

The sensitivity and specificity of serum IL-6 level 4.43 pg/mL above is illustrated as the receiver operating characteristic (ROC) curve as seen in Figure 1 with ROC 0.87.

**DISCUSSION**

Interleukin-6 is known as a proinflammatory cytokine that could stimulate T-lymphocyte and B-lymphocyte, and stimulate acute phase proteins such as hsCRP in the liver.$^{18}$ The IL-6 is induced by various stimulations, such as tumor necrosis factor, IL-1, interferon-$\gamma$, virus, and bacterial endotoxin. The IL-6 is expressed at the shoulder of athero-
sclerotic plaque and is able to increase plaque instability through the metalloproteinase matrix expression, the MCP-1 and TNF-α.\textsuperscript{19}

In this study the serum IL-6 level in ACS is higher compared to CHD. This reveals the role of IL-6 in the pathophysiology of ACS with strong positive correlation with hsCRP level ($r=0.80$, $p=0.00$) (data not shown). This correlation also shows an increase of acute phase protein through cytokine inflammation pathway. Several studies have found serum IL-6 level to be higher in UAP\textsuperscript{9,10,20} and AMI\textsuperscript{21,10} compared to CHD patients.

Biasucci et al\textsuperscript{9} reported that in UAP patients, the elevated serum IL-6 level within 48 hours after hospitalization compared to the level on admission, associated with combined end point death, myocardial infarction, and refractory angina.\textsuperscript{9} In the FRISC-II (Fragmin and/or early Revascularization during InStability in Coronary artery disease) study, increased serum IL-6 level (>5 pg/mL) was associated with mortality after 6 and 12 months.\textsuperscript{22} The elevated serum IL-6 level was also identified in patient subgroup who obtained the highest benefit of mortality reduction in early invasive strategy. This shows that elevated serum IL-6 level could identify patients with more severe event index, who receive the benefit with more aggressive treatment.

In order to predict ACS event, the $\geq 4.43$ pg/mL serum IL-6 level cutoff point was obtained, with 80.95% sensitivity and 77.42% specificity. The serum IL-6 level cutoff point was illustrated in the receiver operating characteristic curve as seen in Figure 1. This means that the serum IL-6 level is adequate to predict the possibility of someone to experience acute condition (ACS) when the serum IL-6 level is found to be $\geq 4.43$ pg/mL in CHD.

**CONCLUSION**

Serum IL-6 level in patients with ACS is higher compared to patients with CHD.

Serum IL-6 level with cut-off point $\geq 4.43$ pg/mL could differ ACS from CHD with highest sensitivity and specificity (sensitivity 80.95%, specificity 77.42%, ROC 0.87).

**REFERENCES**


