Diagnostic Test of Serum Pregnancy-Associated Plasma Protein-A Level as Biomarker for Early Diagnosis of Acute Myocardial Infarction

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ABSTRACT

Acute coronary syndrome is the primary cause of death from heart disease worldwide. This syndrome is caused by ruptured/eroded coronary atherosclerotic plaque, resulting in partial/total occlusion of thrombosis. It is necessary to find novel cardiac biomarkers for the identification of plaque progression before ischemic and myocardial necrosis events. Pregnancy Associated Plasma Protein-A (PAPP-A) is an atherosclerotic mediator proven to be a biomarker for plaque instability. This study aimed to determine the performance of serum PAPP-A as a biomarker for the early diagnosis of AMI. This research was an analytical observational study with a cross-sectional approach. Serum PAPP-A was measured using enzyme-linked immunosorbent assay in 82 new patients. They had ACS and were admitted to the emergency installation of Dr. Moewardi Hospital in Surakarta in August-September 2019. The subjects were grouped into the AMI group (NSTEMI and STEMI) consisting of 49(59.8%) subjects and non-AMI (UAP) group composed of 33(40.2%) subjects based on ACS diagnostic criteria of PERKI 2018. Receiver Operator Characteristic (ROC) curve analysis showed that PAPP-A was a good discriminator between AMI and non-AMI patients. The area under the curve was 0.968, 95% CI (0.932–1.004), with a sensitivity of 91.8% and specificity of 90.9% (p< 0.05). The cut-off value from the ROC curve was 2,526 ng/mL. Serum PAPP-A level has excellent performance as a biomarker for early diagnosis of AMI. It can also function as a screening instrument for the identification of UAP cases developing into AMI.

Keyword: PAPP-A, acute coronary syndrome, acute myocardial infarction

INTRODUCTION

Acute Coronary Syndrome (ACS) is a group of clinical manifestations due to the disruption of the coronary arteries caused by rupture/erosion of the coronary atherosclerotic plaque. The ruptured/eroded plaque subsequently results in partial or total thrombosis occlusion. This syndrome consists of Acute Myocardial Infarction (AMI) with or without ST-segment elevation and Unstable Angina Pectoris (UAP).1-3

Acute myocardial infarction is the incidence of myocardial necrosis caused by the unstable ischemic syndrome.4 The diagnosis of AMI is based on clinical symptoms such as chest pain, a history of CHD, a typical electrocardiographic (ECG) image, both STEMI and NSTEMI, and increased biomarkers of myocardial necrosis.2,3

Unstable angina pectoris is a spectrum of ACS that involves an imbalance between available supply and the oxygen demand for the myocardium. Complaints of chest pain in UAP arise at a resting state or mild activity, whereas stable angina symptoms appear during heavy activity and disappear with rest. A distinguishing feature between UAP and AMI is the absence of an increase in myocardial necrosis biomarkers, such as troponin, which represents myocardial damage.5

Currently available cardiac marker parameters include mass Creatin Kinase-MB (CK-MB), troponin I/T, and myoglobin. Creatin kinase mass and troponin I/T are biomarkers of myocardial necrosis that are widely used, especially in the diagnosis of myocardial infarction. Troponin I/T is currently the gold standard for establishing AMI diagnoses due to its higher sensitivity and specificity compared to CK-MB mass.6,7

Considering the increase in myocardial necrosis biomarkers of CK-MB mass and troponin I/T within at least 4-6 hours, depending on the extent of myocardial necrosis, this can cause delay/error in diagnosis and treatment of patients with ACS. The incidence of AMI (NSTEMI), which is not accompanied by an increase in myocardial necrosis biomarkers and many UAPs are not recognized as ACS, this results in a misdiagnosis, that AMI treatment strategies cannot be immediately established.3
Measurement of myocardial necrosis biomarkers plays an essential role in establishing the diagnosis and risk stratification of patients with suspected AMI. Myocardial necrosis biomarkers that have good sensitivity and specificity with faster emergence time than CK-MB mass and troponin I/T is needed. The biomarker is used for early enforcement of AMI diagnosis, identifying the development and activity of atherosclerosis plaque before the occurrence of myocardial necrosis, especially for monitoring/screening UAP cases developing to AMI.

Pregnancy-associated plasma protein-A is a zinc-binding metalloprotease, which has the potential for proatherosclerosis and has been shown to be a specific activator of Insulin-Like Growth Factor I (IGF-I), a mediator of atherosclerosis, produced by Vascular Smooth Muscle Cells (VSMC), Endothelial Cells (EC), fibroblasts and macrophages that are activated by the presence of proinflammatory cytokines, especially interleukin-1β (IL-1β) and tumor necrosis factors-α (TNF-α).

Previous studies have shown that PAPP-A is abundantly expressed in eroded and ruptured plaque, but only minimally expressed in stable plaque, and released into the blood circulation immediately after the vulnerable plaque/unstable plaque has ruptured or eroded.

**METHODS**

This study was an observational analytic study with a cross-sectional approach to determine the performance of serum PAPP-A levels as biomarkers for early diagnosis of AMI. The study involved 82 new patients in the emergency room at the Dr. Moewardi Hospital, Surakarta, who were diagnosed with new ACS (UAP, NSTEMI, and STEMI) by clinicians according to PERKI 2018 during August to September 2019.

The inclusion criteria of this study were new ACS patients, aged ≥ 18 years, willing to participate in the research and sign the consent form. Exclusion criteria were patients with a history of heart failure, pregnancy at the time of sampling, post-abortion/postpartum at least one month earlier, malignancy, impaired renal function, impaired liver function, stroke, history of major surgery or trauma of at least three months previously (obtained from the history and medical records), and unqualified sample conditions such as hemolysis, jaundice and lipemic.

Serum PAPP-A measurement was performed using serum samples, human PAPP-A sandwich enzyme-ELISA quantikine reagent kit, and Rayto RT-2100C (microplate reader) at a wavelength of 450 nm. High sensitive troponin I (hs-TnI) and mass CKMB levels were measured with Vidas Biomerieux. Random glucose level, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and hs-CRP levels were measured by Architect i4000 Biorad.

Laboratory tests were preceded by a within-day precision test to ensure the quality of the measurement results. Data on the basic characteristics of research subjects and research variables were expressed in n (%), mean±SD, or median (25th-75th percentile).

Data were analyzed using descriptive analysis. The Kolmogorov-Smirnov statistical test was used to determine the pattern of data distribution because this study involved 82 research subjects. Normally distributed data were stated as mean±SD, whereas abnormally distributed data were stated as medians (25th-75th percentile).

This study performed different tests. Chi-Square test was used on data with a nominal scale such as gender, family history of heart disease, history of DM, history of hypertension, history of dyslipidemia, and history of smoking.

Normalization test in the AMI group (NSTEMI, STEMI) and non-AMI (UAP) were performed with the Shapiro-Wilk test because there were 49 subjects in the AMI group (NSTEMI, STEMI) and 33 subjects in the non-AMI group (UAP). The difference test of an independent T-test was used for normally distributed data. In contrast, the Mann-Whitney test was used for abnormally distributed data to the laboratory parameters in the AMI group (NSTEMI and STEMI) and non-AMI (UAP). Groups were determined based on ACS diagnostic criteria, according to PERKI 2018.

Cut-off parameters of serum PAPP-A were obtained from the ROC and AUC curves before the diagnostic test was performed with a 2x2 table to measure sensitivity, specificity, PPV, NPV, PLR, and NLR for serum PAPP-A levels against diagnostic criteria of ACS patients according to PERKI 2018. Data were statistically analyzed using the SPSS version 16.0 program. The result was significant, with p <0.05 with a 95% CI.

This study was approved by Dr. Moewardi Hospital, Surakarta Research Ethics Committee in Surakarta, with an ethical clearance letter dated August 27, 2019, number 1035/VII/HREC/2019.

**RESULTS AND DISCUSSION**

The study involved 82 patients diagnosed with ACS (UAP, NSTEMI, and STEMI) admitted to the
Intensive Care Unit of Dr. Moewardi Hospital in Surakarta in August-September 2019 who met the inclusion and exclusion criteria and divided into two groups: AMI and non-AMI (UAP), characteristics of research subjects can be seen in Table 1.

Based on the characteristics of the research subjects on Table 1, there were no significant differences (p > 0.05) on variables of gender, age, family history of cardiac disease, history of DM, history of hypertension, history of dyslipidemia, history of smoking, serum total cholesterol levels, serum triglyceride levels, serum HDL cholesterol levels, serum LDL cholesterol levels, the onset of chest pain and BMI between AMI and non-AMI (UAP) patients.

Random blood glucose levels in AMI and non-AMI (UAP) patients showed a significantly different median (p < 0.05). AMI patients showed a higher median of the blood glucose level of 149 (125.5-220) mg/dL compared to that of non-AMI patients of 131 (106.5-187.5) mg/dL. The difference in median blood glucose levels in the two groups was influenced by glucose control in subjects with type 2 DM.

There was a significant difference (p < 0.05) in serum hs-CRP levels between AMI patients with a median value of 2.46 (1.23-3.8) mg/dL and non-AMI patients.

**Table 1.** Characteristics of research subjects

<table>
<thead>
<tr>
<th>Variable (measurement unit)</th>
<th>Total N=82(100%)</th>
<th>AMI n=49 (59.8%)</th>
<th>Non-AMI n=33 (40.2%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender (%)</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60 (73.2%)</td>
<td>38 (77.6%)</td>
<td>22 (66.7%)</td>
<td>0.275</td>
</tr>
<tr>
<td>Female</td>
<td>22 (26.8%)</td>
<td>11 (22.4%)</td>
<td>11 (33.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years old)</strong></td>
<td>58.37±9.6</td>
<td>58.33±10.09</td>
<td>58.42±8.98</td>
<td>0.946</td>
</tr>
<tr>
<td><strong>Medical history</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac disease (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31 (37.8%)</td>
<td>18 (36.7%)</td>
<td>12 (36.4%)</td>
<td>0.973</td>
</tr>
<tr>
<td>DM (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31 (37.8%)</td>
<td>20 (40.8%)</td>
<td>11 (33.3%)</td>
<td>0.493</td>
</tr>
<tr>
<td>Hypertension (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55 (67.1%)</td>
<td>31 (63.3%)</td>
<td>24 (72.7%)</td>
<td>0.233</td>
</tr>
<tr>
<td>Dyslipidemia (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46 (56.1%)</td>
<td>26 (53.1%)</td>
<td>20 (60.6%)</td>
<td>0.342</td>
</tr>
<tr>
<td>Smoking (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50 (61%)</td>
<td>30 (61.2%)</td>
<td>20 (60.6%)</td>
<td>0.471</td>
</tr>
<tr>
<td>Random blood glucose (mg/dL)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>138 (115.5-200.5)</td>
<td>149 (125.5-220)</td>
<td>131 (106.5-187.5)</td>
<td>0.043*</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dL)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>182.09±53.61</td>
<td>176.57±49.17</td>
<td>190.27±59.43</td>
<td>0.259</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dL)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>121,5</td>
<td>121</td>
<td>128</td>
<td>0.702</td>
</tr>
<tr>
<td>Serum HDL (mg/dL)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40</td>
<td>40</td>
<td>41</td>
<td>0.698</td>
</tr>
<tr>
<td>Serum LDL (mg/dL)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>126,79±47.96</td>
<td>118</td>
<td>130</td>
<td>0.321</td>
</tr>
<tr>
<td>Serum hs-CRP (mg/dL)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.02</td>
<td>2.46</td>
<td>0.55</td>
<td>0.0001*</td>
</tr>
<tr>
<td>hs-Tnl (ng/L)</td>
<td>167.1</td>
<td>787.4</td>
<td>4.8</td>
<td>0.0001*</td>
</tr>
<tr>
<td>CK-MB mass (ng/L)</td>
<td>4.69</td>
<td>8.25</td>
<td>1.82</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Onset of chest pain (hour)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.5</td>
<td>6</td>
<td>5</td>
<td>0.669</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>22.11</td>
<td>23.03±2.84</td>
<td>22.25±2.17</td>
<td>0.189</td>
</tr>
</tbody>
</table>

Description:
- DM = Diabetes Melliitus; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; hs-CRP = High Sensitivity C-Reactive Protein; hs-Tnl = High Sensitivity-Troponin I; CK-MB Mass = Creatin Kinase-MB Mass
- a: nominal data, presented in (%), Difference test of Chi-Square test was used, p < 0.05 was significant
- b: normally distributed data, presented as mean±SD, difference test with independent T-test was used, p < 0.05 was significant
- c: abnormally distributed data, presented as median (25<sup>th</sup>-75<sup>th</sup> percentile), difference test of Mann-Whitney test was used, p < 0.05 was significant
serum PAPP-A levels in AMI patients compared to non-AMI patients, showing a significantly higher difference ($p<0.000$) of serum PAPP-A levels between AMI (NSTEMI, STEMI) patients and 1.01 (0.95-1.07) ng/mL was found in non-AMI (UAP) patients. Results of Table 2, the median value of serum PAPP-A of all research subjects was 12.61 (1.03-32.92) ng/mL, with details as follows: median value of 32.11 (22.05-36.44) ng/mL was found in AMI (NSTEMI, STEMI) patients and 1.01 (0.95-1.07) ng/mL was found in non-AMI (UAP) patients [median (25th-75th percentile) 8.25 (4.86-39.1) ng/L] compared to non-AMI (UAP) patients [median (25th-75th percentile) 1.82 (0.94-2.69) ng/L]. The study results were following the definitions and diagnostic criteria of ACS, such as increased mass hs-TnI and CK-MB levels in AMI patients (STEMI and NSTEMI) biomarkers of myocardial necrosis. The median of hs-TnI and CK-MB mass of non-AMI (UAP) patients was much lower than the group of AMI patients, that the levels did not exceed the ULs of hs-TnI and CK-MB.4

According to characteristics of research variables in Table 2, the median value of serum PAPP-A of all research subjects was 12.61 (1.03-32.92) ng/mL, with details as follows: median value of 32.11 (22.05-36.44) ng/mL was found in AMI (NSTEMI, STEMI) patients and 1.01 (0.95-1.07) ng/mL was found in non-AMI (UAP) patients. Results of Mann-Whitney test showed a very significant difference ($p<0.000$) of serum PAPP-A levels between AMI (NSTEMI, STEMI) patients and non-AMI patients, showing a significantly higher serum PAPP-A levels in AMI patients compared to non-AMI (UAP) patients.

Table 2. Characteristics of research variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n=82)</th>
<th>AMI (n=49)</th>
<th>Non-AMI (n=33)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP-A levels (ng/mL)</td>
<td>12.61 (1.03-32.92)</td>
<td>32.11 (22.05-36.44)</td>
<td>1.01 (0.95-1.07)</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

Description:
PAPP-A = Pregnancy-Associated Plasma Protein-A; mg/dL=miligram per desilitre; ng/L=nanogram per litre. Data were presented as median (25th-75th percentile), difference test of Mann-Whitney test was used, $p<0.05$ was significant.
serum PAPPA levels was 2,526 ng/mL with an AUC value of 0.968 and a 95% confidence interval (0.932-1.004).

![ROC Curve](image)

**Figure 1.** ROC curve of serum PAPPA levels in AMI incidence

Results of diagnostic tests with a cut-off of 2,526 ng/mL for serum PAPPA levels are shown in Table 3. Serum PAPPA levels ≥ 2,526 ng/mL were identified as positive AMI, and levels < 2,526 ng/mL were identified as negative (non-AMI).

Table 2x2 of the diagnostic test shows that 48 patients (58.54%) out of a total of 82 subjects had serum PAPPA levels ≥ 2,526 ng/mL, consisting of 45 patients (54.88%) positively diagnosed with AMI and three patients (3.66%) diagnosed as non-AMI (UAP). From a total of 34 patients (41.46%) with serum PAPPA levels < 2,526 ng/mL, it was found that four patients (4.88%) were positively diagnosed with AMI and 30 patients (36.58%) were negative AMI and diagnosed non-AMI (UAP) instead (Table 3).

From the results of diagnostic tests, the performance of serum PAPPA levels as biomarkers for early diagnosis of AMI was as follows: sensitivity of 91.8%; specificity of 90.9%; PPV of 93.8%; NPV of 88.2%; PLR of 10.1; and NLR of 0.1 with an AUC value of 0.968 CI 95% (0.932-1.004).

The diagnostic test results above show that serum PAPPA levels have excellent performance as an early AMI biomarker because they have outstanding AUC values (> 0.9-1). The sensitivity and specificity of serum PAPPA > 90% indicate that serum PAPPA can function as a screening instrument and its use as an early diagnostic instrument. The higher sensitivity values compared to specificity enable serum PAPPA levels to be a screening biomarker, which can identify AMI cases and screen UAP patients who develop into myocardial infarction (NSTEMI or STEMI). The diagnostic value of a laboratory test is considered good if the PLR value is almost ten, and the NLR is nearly 0. Serum PAPPA levels in this study has an excellent diagnostic value for early diagnosis of AMI because it has a PLR value of 10.1 and an NLR value of 0.1.

The characteristics of the subjects of this study also support the research by Wang et al., which presented a table of risk factors related to ACS in the study subjects. It was mentioned that there was no significant difference in age, family history of CHD, hypertension, hyperlipidemia, DM, smoking, sedentary lifestyle, and BMI between the ACS groups and the healthy control group. The study also found significant differences in serum glucose levels, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and diastolic blood pressure between ACS patients and healthy control groups, with the higher mean value of the parameters in ACS patients compared with healthy patients.²

This study also supports research by Gururajan et al. The characteristics table of the study subjects showed that there was no significant difference in mean values of serum glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and diastolic blood pressure between ACS patients and healthy control groups. However, there was a significant difference if compared to the healthy control and Non-Cardiac Chest Pain (NCCP).¹⁰

The results of this study indicated that serum PAPPA levels in AMI (NSTEMI and STEMI) patients were much higher than in non-AMI (UAP) patients,

Table 3. Table 2x2 of the diagnostic test of PAPPA to ACS diagnostic criteria

<table>
<thead>
<tr>
<th>Serum PAPPA (ng/mL)</th>
<th>AMI Criteria (gold standard)</th>
<th>Non-AMI Criteria</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (≥ 2,526)</td>
<td>AMI n (%) Positive</td>
<td>Non-AMI n (%) Negative</td>
<td></td>
</tr>
<tr>
<td>45 (54.88%)</td>
<td>3 (3.66%)</td>
<td></td>
<td>48 (58.54%)</td>
</tr>
<tr>
<td>Negative (&lt; 2,526)</td>
<td>4 (4.88%)</td>
<td>30 (36.58%)</td>
<td>34 (41.46%)</td>
</tr>
<tr>
<td>49 (59.76%)</td>
<td>33 (40.24%)</td>
<td></td>
<td>82 (100%)</td>
</tr>
</tbody>
</table>

Note: PAPPA=Pregnancy-Associated Plasma Protein-A, AMI=Acute Myocardial Infarction

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Diagnostic Test of Serum Pregnancy - Astuti, et al.
despite no increased hs-TnI levels were found in some AMI patients, showing a similar result to the research by Bayes et al.\(^7\)

The study by Heeschen et al. and Iversen et al. showed that there was no significant difference in mean serum PAPP-A levels between NSTEMI and UAP patients. Still, there was a significant difference in STEMI patients compared to NSTEMI patients and UAP. Those findings were contradictory with the results in that serum PAPP-A levels in AMI (NSTEMI and STEMI) were much higher than serum PAPP-A levels in non-AMI (UAP) patients.\(^{15}\)

**CONCLUSIONS AND SUGGESTIONS**

Measurement of serum PAPP-A levels at a cut-off of 2,526 ng/mL has an excellent diagnostic test performance as a biomarker for early diagnosis of AMI. It can also function as a screening instrument to identify UAP cases developing into AMI (NSTEMI and STEMI), with a sensitivity of 91.8%; specificity of 90.9%; PPV of 93.8%; NPV of 88.2%; PLR of 10.1; NLR of 0.1 with an AUC value of 0.968 and CI 95% (0.932 - 1.004).

Further research with prospective cohort design was needed by involving angina pectoris patients diagnosed with UAP as research subjects and additional subjects as healthy control. They performed a diagnostic test of serum PAPP-A levels by comparison and combination with other cardiac biomarkers such as myocardial necrosis and inflammation.

**REFERENCES**