Value and Level of Galectin-3 in Acute Myocardial Infarction Patients Undergoing Primary Percutaneous Coronary Intervention

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Aim: This study investigated the impact of the circulating galectin-3 level on the 30-day prognostic outcome in patients with ST-segment elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (PCI).

Methods: From May 2009 to March 2011, blood samples for assessment of the circulating galectin-3 level were collected from 196 consecutive STEMI patients treated by primary PCI and from 30 healthy volunteers.

Results: The galectin-3 level was determined using ELISA. Our results demonstrated that the circulating level of galectin-3 was significantly higher in STEMI patients than in healthy control subjects ($p<0.001$). As compared with patients with galectin-3 <7.67 ng/mL, patients with galectin-3 ≥7.67 ng/mL were significantly older, had significantly lower left ventricular ejection fraction and significantly higher frequency of elevated white blood cell count, advanced Killip score (defined as ≥ score 3), congestive heart failure (defined as ≥ New York Heart Association Functional Class III), respiratory failure, unstable hemodynamics requiring a mechanical ventilator and intra-aortic balloon pump support, multiple vessel diseases and 30-day mortality (all $p<0.04$). Furthermore, multivariate analysis showed that elevated circulating level of galectin-3 was the strongest independent predictor of the combined 30-day major adverse clinical outcome (MACO) (defined as advanced CHF or 30-day mortality) ($p<0.0001$).

Conclusion: A high circulating galectin-3 level may serve as a useful biomarker for predicting 30-day MACO in patients with STEMI undergoing primary PCI.


Key words: Acute myocardial infarction, Galectin-3 level, Primary percutaneous coronary intervention, Major adverse clinical outcome

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Introduction

Despite state-of-the art treatment, acute myocardial infarction (AMI) is still a progressive disorder characterized by high morbidity and mortality¹-⁶, suggesting that important pathogenic mechanisms remain active and unmodified by current treatment⁵, ⁶. Prior
clinical studies have reported that the occurrence of congestive heart failure (CHF) may denote a poor prognostic outcome in patients with decompensated cardiovascular diseases4,7-9). Many reports have also suggested a link between the biomarkers and CHF to short-term and long-term prognostic outcomes in AMI patients10-14). Of these biomarkers, the circulating level of galectin-3 has emerged as a novel informative biomarker on the prediction of fibrosis15), cardiac dysfunction6), cardiac remodeling16), and the development and progression of heart failure17). Furthermore, galectin-3 has been described as a useful indicator for identifying CHF patients with high risk of readmission or death17); however, the impact of the circulating level of galectin-3 on the prognostic outcome of STEMI patients undergoing primary PCI has not been well addressed.

Galectins has been identified to be a family of lectins that bind β-galactosides18,19). The expression of galectin-3 has been detected in leukocytes, mast cells20,21) and various organ tissues22). Over-expression of galectin-3 has been observed in patients with decompensated CHF6). Involvement of galectin-3 in myofibroblast activation with resultant hepatic fibrosis15) and the promotion of renal fibrosis23) has also been described. In addition, galectin-3 may play an important role in the inflammatory response, fibrosis and scar formation, cardiac remodeling and heart failure in the clinical setting of AMI16,24-27). The purpose of the present study was to verify the hypothesis that an elevated circulating galectin-3 level is a useful biomarker for predicting the major adverse clinical outcome (MACO) in patients with STEMI undergoing primary PCI.

Materials and Methods

Patient Population, Inclusion and Exclusion Criteria

All patients with acute STEMI are considered eligible for primary PCI at our institute. In order to circumvent other potential influences on the circulating level of galectin-3, patients with documented histories of recent surgery, intracranial hemorrhage, trauma or myocardial infarction within the preceding 2 months, malignancies, febrile disorders, acute or chronic inflammatory diseases, hematologic disorders and autoimmune diseases with or without immunosuppressive therapy were excluded from the present study.

From May 2009 to March 2011, 196 patients presenting with STEMI of <12 hours’ duration with primary PCI were recruited. Blood samples for assessment of the circulating galectin-3 level were collected via the antecubital vein and stored in heparinized test-tubes 6 hours after the PCI procedure. The blood sample was collected during this time interval because the peak level of CK-MB and many inflammatory biomarkers will appear within 6 to 12 h after acute inflammatory stimulation. Additionally, the blood sample was collected 6 hours after PCI procedure based on the results of a previous experimental study28), which showed that the accumulation of galectin-3 peaked 12 hours after acute inflammatory stimulation.

Blood samples were also obtained from 30 age- and gender-matched healthy volunteers during a health check in the Health-Promotion Center as normal controls. Informed consent was obtained from each study subject.

Procedure and Protocol

At our hospital, a transradial artery approach using a 6-French arterial sheath is routinely applied for the treatment of AMI unless both hands are positive for the Allen test. A 6-French Kimny Miniradi (Boston Scientific, Scimed Inc., Maple Grove, MN) was used for diagnostic studies and primary PCI. Intra-aortic balloon pump (IABP) support was performed with a right femoral arterial approach in patients with acute pulmonary edema associated with an unstable condition or hemodynamic instability. When necessary, the PercuSurge GuardWire device ((Medtronic AVE) was also utilized and the inclusion and exclusion criteria of the procedure have been described previously29).

Clopidogrel (600 mg loading dose before stenting, then 75 mg/day) was given to patients who underwent primary stenting. Unless contraindicated, aspirin (100 mg orally, once a day) was routinely administered. Other commonly prescribed medications included angiotensin-converting enzyme inhibitors (ACEIs), beta-blockers, isorotinate and diuretic drugs.

Blood Sample Processing and Laboratory Investigations

After centrifugalation, the samples were stored at −80°C before the assay of the galectin-3 level. White blood cell (WBC) counts, biochemical measurements and electrolyte levels were performed with standard laboratory methods.

Serum galectin-3 was measured in duplicate with a commercially available ELISA method (R & D). The intra-observer variability of the measurements of galectin-3 was also assessed and the mean intra-assay coefficients of variance were all <4.6%.
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Analysis was used for assessing independent predictors of the 30-day occurrence of MACO. Statistical analysis was performed using SPSS statistical software for Windows version 13 (SPSS for Windows, version 13; SPSS Inc., IL, USA). A p value of <0.05 was considered significant.

Results

Baseline Characteristics of the Patients with STEMI and Healthy Volunteers (Table 1)

Table 1 summarizes the baseline characteristics of
The patients with STEMI and healthy volunteers. The patients with STEMI and normal controls showed no significant differences with respect to age, gender, and the levels of total cholesterol and low-density lipoprotein; however, the WBC count and the levels of creatinine and galectin-3 were significant higher in STEMI patients than in normal controls.

More than 55% STEMI patients had a history of current smoking and hypertension. On the other hand, less than 10% STEMI patients had a history of previous myocardial infarction and old stroke; however, none of the healthy volunteers had these histories.

**Comparison of Baseline Variables, Laboratory Findings, Clinical Presentations, and 30-Day Outcomes in Patients with High and Low Circulating Galectin-3 Level (Fig. 1 and 2 and Table 2)**

Receiver operating characteristics (ROC) curve analysis (Fig. 1) revealed that a circulating level of galectin-3 ≥ 7.67 ng/mL was the most powerful predictor of 30-day MACO with sensitivity of 74.5%, specificity of 72.4%, a positive predictive value of 61.3% and a predictive value of 89.1%. For further analysis (Table 2), patients with high circulating galectin-3 (≥ 7.67 ng/mL) were designated as group 1 and patients with low circulating galectin-3 (< 7.67 ng/mL) were designated as group 2.

In order to determine whether a circulating level of galectin-3 ≥ 7.67 ng/mL was also a powerful predictor of severe left ventricular dysfunction [defined as left ventricular ejection fraction (LVEF) < 40%], ROC curve analysis was performed and the results showed that it was a significantly powerful predictor with a sensitivity of 62.8% and specificity of 81.7%, respectively (Fig. 2-A).

Similarly, to elucidate whether a circulating level of galectin-3 ≥ 7.67 ng/mL was also a powerful predictor of higher circulating level of CPK (defined as > 3000 IU/L), ROC curve analysis was performed. As expected, the results showed that a circulating level of

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (n=78)*</th>
<th>Group 2 (n=118)*</th>
<th>p value§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>66.6 ± 10.8</td>
<td>57.7 ± 11.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender</td>
<td>70.5% (55)</td>
<td>90.7% (107)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>56.2% (41)</td>
<td>22% (26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>64.5% (51)</td>
<td>49.2% (58)</td>
<td>0.025</td>
</tr>
<tr>
<td>Current smoking</td>
<td>44.9% (35)</td>
<td>67.8% (80)</td>
<td>0.002</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>5.1% (4)</td>
<td>11.9% (14)</td>
<td>0.178</td>
</tr>
<tr>
<td>Old stroke</td>
<td>11.5% (5)</td>
<td>5.9% (7)</td>
<td>0.256</td>
</tr>
<tr>
<td>Infarction location by ECG</td>
<td></td>
<td></td>
<td>0.543</td>
</tr>
<tr>
<td>Anterior wall</td>
<td>66.7% (52)</td>
<td>57.6% (68)</td>
<td></td>
</tr>
<tr>
<td>Non-anterior wall</td>
<td>33.3% (26)</td>
<td>42.4% (50)</td>
<td></td>
</tr>
<tr>
<td>WBC count (×10³/dL)</td>
<td>11.8 ± 4.3</td>
<td>10.4 ± 3.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Creatinine level (mg/dL)</td>
<td>1.81 ± 2.47</td>
<td>1.15 ± 1.17</td>
<td>0.013</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>176 ± 46</td>
<td>188 ± 43</td>
<td>0.142</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>116 ± 42</td>
<td>120 ± 39</td>
<td>0.539</td>
</tr>
<tr>
<td>Peak CPK level (IU/L)</td>
<td>2192 ± 2318</td>
<td>1675 ± 1579</td>
<td>0.056</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>54.3 ± 14.9</td>
<td>59.0 ± 11.8</td>
<td>0.014</td>
</tr>
<tr>
<td>Mechanical ventilator support</td>
<td>14.1% (11)</td>
<td>4.2% (5)</td>
<td>0.028</td>
</tr>
<tr>
<td>Intra-aortic balloon pump support</td>
<td>28.2% (22)</td>
<td>10.2% (10)</td>
<td>0.002</td>
</tr>
<tr>
<td>Advanced Killip score‡</td>
<td>48.7% (38)</td>
<td>10.2% (12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Advanced CHF¶</td>
<td>35.9% (28)</td>
<td>8.5% (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>7.7% (6)</td>
<td>0.8% (1)</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD or % (No.) of patients.
CHF = congestive heart failure; CPK = creatine phosphokinase; ECG = electrocardiogram; LVEF = left ventricular ejection fraction; WBC = white blood cell.
*Group 1 = high galectin-3 (≥ 7.67 ng/mL); Group 2 = low galectin-3 (< 7.67 ng/mL)
†indicates data obtained by 2-D echocardiographic examination on day-2 following AMI.
‡defined as Killip score ≥ 3.
¶defined as New York Heart Association Functional classification ≥ 3.
§Indicated continuous variables were analyzed using the independent t test, and categorical variables with the Chi-square test.
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TIMI-3 flow; however, the patients in group 1 had a significantly higher frequency of multiple vessel disease than the patients in group 2.

Linear Regression Analysis of the Correlation of the Galectin-3 Level to Killip Score, WBC Count, Creatinine Level, Multiple Vessel Disease, and LVEF (Fig. 3)

Linear regression analysis revealed significant positive correlations of the circulating galectin-3 level to the Killip score on presentation \( (r=0.427, p<0.001) \), WBC count \( (r=0.260, p<0.001) \) and creatinine level \( (r=0.225, p=0.002) \) but a significant negative correlation to LVEF \( (r=-0.253, p<0.001) \). Additionally, analysis also revealed significant positive correlations of the number of vessel diseases to circulating galectin-3 \( [r=0.203, p=0.004] \) and WBC count \( [r=0.183, p=0.017] \).

Spearman Rank Correlation (R) Analysis between Circulating Galectin-3 Level and Advanced CHF, Unstable Hemodynamics Requiring IABP Support and CADILLAC Risk Score (Table 4)

Spearman rank correlation analysis revealed significant correlations between the circulating level of galectin-3 and advanced CHF, unstable hemodynamic requiring IABP support and the CADIALC risk score \(^{30}\).

Correlation (R) between Level of Circulating Galectin-3 and CADILLAC Risk Score

The correlation between the circulating galec-
ties variables were evaluated and multiple stepwise logistic regression analysis revealed that high galectin-3 \((\geq 7.67 \text{ ng/mL})\), multiple vessel disease, low LVEF, and a high creatinine level were significant independent predictors of 30-day MACO among patients with STEMI undergoing primary PCI.

### Discussion

The creatinine level\(^{30,32}\), LVEF\(^{7,12}\) and multiple vessel disease\(^{33}\) are three traditional risk factors for predicting an unfavorable clinical outcome after AMI and the present study confirmed that these three traditional risk factors were also significant independent predictors of 30-day MACO in patients with STEMI undergoing primary PCI. In addition to traditional risk factors, many biomarkers have been linked to unfavorable clinical outcomes\(^{10-14}\) of AMI-induced CHF. Recently, galectin-3 has been reported to be a novel mediator which may participate in the development and progression of CHF\(^{16,17}\); however, the role of the circulating galectin-3 level in the clinical outcome prediction of patients with STEMI undergoing primary PCI needs further investigation.

### Univariate Analysis of Baseline Variables, Clinical Features and Angiographic Findings for Predicting 30-Day Major Adverse Clinical Outcomes (Table 5)

After univariate analysis of the variables in Tables 2 and 3 for the prediction of 30-day MACO, the data of the significant variables are summarized in Table 5. Significant predictors of 30-day MACO included high galectin-3 \((\geq 7.67 \text{ ng/mL})\), old age, diabetes mellitus, multiple vessel disease, low LVEF, high WBC count, creatinine level, and peak level of CPK.

### Independent Predictors of 30-Day MACO (Table 6)

A wide variety of baseline clinical and laborato-
Fig. 3. Highly significant correlation between the circulating galectin-3 level and Killip scores on presentation (A). Relatively weak but still significant correlation between the circulating galectin-3 level and white blood cell count (B). Also, relatively weak but still significant correlation between the circulating galectin-3 level and creatinine level (C). Conversely, a significant negative correlation between the circulating galectin-3 level and left ventricular ejection fraction (LVEF) (D). Additionally, the analysis also revealed a relatively weak but still significant correlation of the number of vessel disease to WBC count (E) and circulating galectin-3 (F).
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Table 4. Spearman Rank Correlation (R) between Serum Galectin-3 and Advanced CHF, Unstable Hemodynamics Required IABP Support and CADILLAC Risk Score

<table>
<thead>
<tr>
<th>Variables</th>
<th>R*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced CHF and serum galectin-3</td>
<td>0.457</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Unstable hemodynamics and serum galectin-3†</td>
<td>0.361</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CADILLAC risk score and serum galectin-3</td>
<td>0.512</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CHF = congestive heart failure; TIMI = thrombolysis in myocardial infarction; †Spearman rank correlation.

Table 5. Univariate Analysis of Baseline Variables in Prediction of 30-Day Major Adverse Clinical Outcome (MACO)*

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>1.057</td>
<td>1.027-1.088</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.109</td>
<td>1.095-4.060</td>
<td>0.026</td>
</tr>
<tr>
<td>WBC (×10^3/dL)</td>
<td>1.115</td>
<td>1.025-1.213</td>
<td>0.011</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.448</td>
<td>1.098-1.909</td>
<td>0.009</td>
</tr>
<tr>
<td>Peak CKP level (IU/L)</td>
<td>1.024</td>
<td>1.007-1.041</td>
<td>0.005</td>
</tr>
<tr>
<td>High galectin-3 level (≥7.67 vs. &lt;7.67 ng/mL)</td>
<td>11.364</td>
<td>7.673-15.882</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Multiple vessel disease†</td>
<td>4.773</td>
<td>2.222-10.252</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>0.910</td>
<td>0.876-0.945</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

LVEF = left ventricular ejection fraction; CKP = creatine phosphokinase; WBC = white blood cell.

* major adverse clinical outcome (MACO) defined as composite endpoint of advanced congestive heart failure or 30-day mortality.
† defined as obstruction of ≥50% of epicardial vessel.

primary PCI is currently unclear. The present study suggests several important clinical implications of the circulating galectin-3 level in such clinical conditions.

First, our study verifies that circulating galectin-3 was significantly higher in AMI patients than in normal controls. Inflammation and oxidative stress34-36 play a key role in all stages of atherosclerosis and vascular remodeling, from initiation, progression of athemaurus plaques and luminal narrowing, finally leading to acute coronary syndromes11, 31 as a consequence of plaque rupture. Interestingly, galectin-3 has been shown to be an important contributor to inflammation6, 16. In the present study, compared with the healthy controls, the circulating galectin-3 level and WBC count, an index of inflammation, were significantly higher in STEMI patients. Furthermore, multiple vessel disease, an indirect index of a higher degree of inflammation, was significantly more frequently seen in patients with high galectin-3 than in those with low galectin-3. In addition, linear regression analysis revealed significant positive correlations of multiple vessel disease to the circulating level of galectin-3 and WBC count. These findings could partially explain the impact of inflammation on multiple vessel disease, progression of athermanous plaques (i.e., multiple plaque in coronary artery tree), and plaque rupture/STEMI in our patients. Of importance was that these findings imply that galectin-3 may, at least in part, participate in inflammatory reactions and atherosclerosis in patients presenting with STEMI.

Second, there were significant positive correlations of high circulating galectin-3 to an advanced Killip score, unstable hemodynamics requiring IABP support, advanced CHF and a high CADILLAC risk score. Conversely, a significant negative correlation between the galectin-3 level and LVEF was also noted. Such correlations imply that elevated circulating galectin-3 may be an indirect indicator of left ventricular dysfunction and pump failure following AMI.

Third, the present study demonstrated that there were strong correlations between elevated galectin-3 and the WBC count and creatinine level, two important indices of an unfavorable clinical outcome, in STEMI patients after primary PCI. Most importantly, elevated circulating galectin-3 was proven to be a strong independent predictor of 30-day MACO among patients with STEMI undergoing primary PCI and thus can be utilized as a useful biomarker for stratifying high and low risk subgroups in daily clinical practice.
Limitations

The current study has several limitations. First, this study did not assess serial changes in the circulating level of galectin-3 after STEMI; therefore, the best timing for measuring the peak level of galectin-3 in patients after STEMI could not be identified. Second, this study did not measure the time interval of galectin-3 levels prior to and just after the PCI procedure. Thus, the impact of balloon dilation during PCI on stimulating the release of galectin-3 from the vessel wall remains uncertain. Third, the role of the circulating galectin-3 level for predicting the long-term clinical outcome of patients with STEMI undergoing primary PCI remains unclear; therefore, further large series studies and more experiments for evaluating the time course of serum galectin-3 level with respect to long-term follow-up is needed. Finally, we did not measure the circulating levels of high-sensitivity C-reactive protein and brain natriuretic peptide, which are two well-known biomarkers predicting the prognostic outcome of STEMI and CHF. Thus, we remain uncertain whether these two biomarkers, which are similar to the circulating galectin-3 level, could influence the morbidity/mortality of our patients.

Conclusion

The present study demonstrated that elevated circulating galectin-3 was a strong independent predictor of 30-day MACO among patients with STEMI undergoing primary PCI and thus can be utilized as a useful biomarker for stratifying high and low risk subgroups in daily clinical practice.

Conflict of Interest

None.

References


Table 6. Multiple Stepwise Logistic Regression Analysis of Predictors for 30-Day Major Adverse Clinical Outcome*  

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.403</td>
<td>1.112-1.771</td>
<td>0.004</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>0.925</td>
<td>0.839-0.958</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Multiple vessel disease †</td>
<td>4.497</td>
<td>1.756-11.518</td>
<td>0.002</td>
</tr>
<tr>
<td>Higher galectin-3 level (≥7.67 vs. &lt;7.67 ng/mL)</td>
<td>5.484</td>
<td>2.385-12.606</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Cl = confidence interval; LVEF = left ventricular ejection fraction; OR = odds ratio.

* major adverse clinical outcome (MACO) defined as composite endpoint of advanced congestive heart failure or 30-day mortality.
† defined as obstruction of ≥50% of epicardial vessel.
come in acute decompensated heart failure. Crit Care, 2011; 15: R1