Inflammatory biomarkers have been proposed for use in the risk stratification of patients with acute myocardial infarction (AMI). We examined the value of inflammatory biomarkers over clinical features for predicting cardiovascular (CV) events in stable outpatients with MI. We enrolled 430 post-MI patients and measured their levels of high-sensitivity C reactive protein (hs-CRP), growth differentiation factor-15 (GDF-15), and the interleukin-1 receptor family member called ST2 (ST2), one month after AMI. Patients were prospectively followed for 3 years. In our study cohort (mean age, 66 ± 12 years; left ventricular ejection fraction, 55 ± 13%), CV events were observed in 39 patients (9.1%). Kaplan–Meier analysis revealed that patients with high levels of GDF-15 (≥1221.0 ng/L) showed poorer prognoses than those with low levels of GDF-15 (<1221.0 ng/L) (20.4% versus 3.6%, P < 0.001); hs-CRP and ST2 did not show a similar correlation with prognoses. GDF-15 remained associated with CV events after adjusting for age, chronic kidney disease, and B-type natriuretic peptide (hazard ratio, 1.001; 95% confidence interval, 1.000 – 1.001; P = 0.046). GDF-15 provided an incremental predictive value for CV events over clinical features (incremental value in global χ² = 43.81, P < 0.001). In outpatients with prior MI, GDF-15 was an independent indicator of CV events, unlike hs-CRP and ST2. GDF-15 provided an incremental prognostic value over clinical features. (Int Heart J 2016; 57: 11-17)

Key words: Coronary artery disease, Growth differentiation factor-15, Prognosis

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The aims of this study were to 1) compare the prognostic significance of inflammatory biomarkers for predicting CV events in clinically stable outpatients with histories of prior MI and 2) to determine if a combined analysis of these inflammatory biomarkers would improve risk stratification beyond that provided by clinical features and BNP levels.

Methods

Study population: This study was a sub-analysis of the assessment of lipophilic versus hydrophilic statin therapy in AMI (ALPS-AMI) study, a prospective, multicenter study comparing the effectiveness of lipophilic atorvastatin and hydrophilic pravastatin for secondary prevention of CV events after AMI; details of the methods and design were previously published. Briefly, patients were randomly allocated to receive either atorvastatin (264 patients) or pravastatin (261 patients) within 96 hours of a primary percutaneous coronary intervention. The inclusion criteria were age > 20 years, a serum low-density lipoprotein-cholesterol level (LDL) > 70 mg/dL, and percutaneous coronary intervention to treat AMI performed up...
to 96 hours prior to enrollment. Exclusion criteria were a planned surgery for coronary artery bypass grafting, pregnancy, active liver or renal disease, malignancy, withdrawal of informed consent, serious arrhythmic events, or the presence of hemodynamic instabilities (hypotension, congestive heart failure, or mechanical complications following AMI). Patients were enrolled between June 2008 and December 2010. In the present study, we screened 430 outpatients, who provided blood samples one month after AMI. The institutional review board approved the protocol, which was registered with the University Hospital Medical Information Network (UMIN 000001521), and written informed consent was obtained from each patient before enrollment. This study was performed in accordance with the Declaration of Helsinki.

**Biomarker measurement:** Venous blood samples, one month after AMI, were collected in blood collection tubes containing citrate-ethylenediamine tetracetic acid. After centrifugation, samples were frozen at -80°C until assayed in a blinded fashion in a dedicated core laboratory. The measured biomarkers included BNP, hs-CRP, GDF-15, and ST2. BNP was measured with a commercially available kit (Tosoh, Tokyo); hs-CRP was measured using an ultrasensitive, particle-enhanced immunoturbidimetric assay (Siemens, München, Germany); GDF-15 was measured using a Quantikine® enzyme-linked immunoassay (R&D Systems, Minneapolis, MN, USA); and ST2 was measured using a Quantikine® enzyme-linked immunosorbent, human ST2/IL-1 R4 immunoassay (R&D Systems). All biomarker measurements were performed by an investigator who was not aware of the patient characteristics and outcomes.

**Study definition and endpoint:** AMI was diagnosed according to the AHA/ACC guidelines. Patients with systolic blood pressure > 140 mmHg and/or diastolic pressure > 90 mmHg and those taking antihypertensive agents were considered to have hypertension. Dyslipidemia was defined as serum LDL-C > 140 mg/dL, high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL, or the requirement of treatment with lipid-lowering agents. Diabetes mellitus was defined as a fasting glucose level ≥ 126 mg/dL or clinical history of oral hypoglycemic agent use or insulin use. Estimated glomerular filtration rate was calculated using the Modification of Diet and Renal Disease equation coefficients modified for Japanese patients. Left ventricular ejection fraction was calculated using the biplane Simpson method from the apical 4- and 2-chamber views. All patients were prospectively followed for 3 years. The study endpoint was the recording of a major adverse cardiovascular event (MACE), including all-cause death, MI, stroke, or hospitalization due to congestive heart failure; patients were divided into an event-free group and a MACE group. We investigated whether inflammatory biomarkers improved MACE risk stratification in outpatients with prior MI.

**Statistical analysis:** Continuous variables are summarized as the mean ± standard deviation if normally distributed, or as median and interquartile range otherwise. Normality was evaluated using the Shapiro–Wilk W-test. Comparisons of baseline categorical data between the two groups were analyzed using the two-sided chi-squared test, whereas differences between continuous variables were compared using the unpaired t-test or the Mann–Whitney U-test. Correlations between the inflammatory biomarkers and other continuous baseline variables were studied using a nonparametric (Spearman’s) correlation coefficient. The optimal receiver-operating characteristics (ROC) curve cut-off value for MACE prediction was chosen as the value maximizing sensitivity and specificity. Kaplan–Meier curves were calculated from the AMI onset to the MACE, and compared using the log-rank test. A Cox proportional hazards regression analysis was performed to identify the MACE predictors using variables that included clinical characteristics, risk factors, and biomarker values. Multivariate analysis was performed using all variables with a P-value < 0.1 in the univariate analysis. The incremental values of BNP and inflammatory biomarkers over the baseline clinical features were assessed using comparisons of model chi-square analyses at each step. A P-value < 0.05 was considered to indicate statistical significance. All analyses were performed using SPSS version 21.0 (SPSS, Chicago, IL, USA) and MedCalc version 12.3.0 (MedCalc Software, Mariakerke, Belgium).

**RESULTS**

**Patient characteristics:** A total of 430 outpatients were enrolled in this study; no patients dropped out and all patients completed the follow-up. During the 3-year follow-up period, adverse events were observed in 39 patients (9.1%), including deaths (n = 26), MIs (n = 4), strokes (n = 5), and hospitalizations due to uncomplicated heart failure (n = 12). The baseline clinical characteristics are listed in Table I. Patients with MACE were older than those without (P = 0.048), and patients’ histories of chronic kidney disease and multi-vessel disease were significantly higher in patients with MACE than in those without. Gender, blood pressure, history of hypertension, diabetes mellitus, and atrial fibrillation were similar between patients with and without MACE. At discharge, the use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and beta-blockers were similar between the two groups. LV ejection fraction was not different between the two groups. BNP levels were higher in patients with MACE compared with those in patients without MACE (P < 0.001). With respect to inflammatory biomarkers, hs-CRP and ST2 levels were similar between the two groups, whereas GDF-15 was significantly higher in patients with MACE than in those without MACE (Figure 1).

GDF-15 showed modest correlation with age, renal function, and BNP levels. The levels of hs-CRP and ST2 were weakly correlated with renal function. The three inflammatory biomarkers weakly correlated with one another (all, r < 0.3) (Table II).

**Predictors of MACE:** Of the biomarkers examined, the area under the ROC curve (AUC) for predicting adverse events was the greatest for GDF-15 (Figure 2). The BNP and GDF-15 AUCs had optimal ROC cut-off points of 134.4 (pg/mL) and 1221.0 (ng/L), respectively. In the Kaplan–Meier analysis, patients with high levels of GDF-15 (≥ 1221.0 ng/L) showed worse prognoses than did those with low levels (< 1221.0 ng/L) (20.4% versus 3.6%, P < 0.001) (Figure 3A); patients with high levels of hs-CRP and ST2 did not show CV event differences. Furthermore, patients with high levels of BNP (≥ 134.4 pg/mL) and GDF-15 had significantly worse prognoses than did patients with low levels of BNP (< 134.4 pg/mL) and low levels of GDF-15, or patients with high BNP levels and low GDF-15 levels, or patients with low BNP levels and high
GDF-15 levels (log-rank test; $P < 0.001$, $P = 0.007$, and $P = 0.007$, respectively) (Figure 3B). In the univariate Cox proportional hazards analysis, age, chronic kidney disease, BNP, and GDF-15 were associated with higher MACE risks. After multivariate Cox proportional hazards analyses, including all variables with $P < 0.1$ in the univariate analysis, high levels of BNP and GDF-15 predicted poor prognoses (Table III). The incremental value for GDF-15 to predict adverse events is shown in Figure 4. BNP levels added to the predictive power over clinical features (incremental value in global $\chi^2 = 36.4$, $P < 0.001$).

| Table I. Baseline Characteristics of Patients With and Without MACE |
|---------------------|---------------------|---------------------|---------------------|---------------------|
| Overall ($n = 430$) | MACE (+) ($n = 39$) | MACE (-) ($n = 391$) | $P$                |
| Age 66 ± 12          | 73 ± 9              | 66 ± 11             | 0.048              |
| Male/Female 343/87   | 309                 | 313/78              | 0.64               |
| Systolic BP (mmHg)   | 133.3 ± 23.8        | 129.5 ± 22.7        | 133.5 ± 23.2       | 0.70               |
| Hypertension, n (%)  | 189 (44)            | 13 (33)             | 176 (45)           | 0.16               |
| Dyslipidemia, n (%)  | 162 (38)            | 13 (33)             | 149 (38)           | 0.10               |
| Diabetes mellitus, n (%) | 104 (24) | 10 (26)             | 94 (24)            | 0.82               |
| Smoking, n (%)       | 278 (65)            | 24 (62)             | 254 (65)           | 0.20               |
| Chronic kidney disease, n (%) | 121 (28) | 19 (49)             | 102 (26)           | 0.0030             |
| Atrial fibrillation, n (%) | 17 (4)      | 3 (8)               | 14 (4)             | 0.22               |
| Multi-vessel, n (%)  | 143 (33)            | 20 (51)             | 123 (31)           | 0.012              |
| ACE-inhibitor/ARB, n (%) | 366 (85) | 33 (85)             | 333 (85)           | 0.93               |
| Beta-blocker, n (%)  | 241 (56)            | 23 (59)             | 218 (56)           | 0.57               |
| Statin, n (%)        | 430 (100)           | 39 (100)            | 391 (100)          | 1.0                |
| eGFR (mL/minute/1.73 m$^2$ surface area) | 70.9 [58.1, 83.6] | 60.0 [41.4, 72.0] | 71.7 [59.6, 84.0] | 0.0010             |
| BNP (pg/mL)          | 85.0 [42.5, 184.2]  | 182.5 [79.9, 344.2] | 75.7 [40.8, 165.4] | < 0.001            |
| LV ejection fraction (%) | 54.7 ± 12.6 | 52.7 ± 10.5         | 55.2 ± 12.8        | 0.36               |

Values are number (%), mean ± standard deviation, or median [25th, 75th percentiles]. ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; CCB, calcium channel blocker; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; hs-CRP, high-sensitivity C reactive protein; LV, left ventricular; MACE, major adverse cardiac events (including all-cause death, myocardial infarction, stroke, or hospitalization due to congestive heart failure); and ST2, interleukin-1 receptor family member called ST2.
Furthermore, GDF-15 provided an incremental predictive value for CV events over clinical features and BNP (incremental value in global $\chi^2 = 43.8$, $P < 0.001$).

**Discussion**

To the best of our knowledge, this is the first report comparing the prognostic significance of inflammatory biomarkers for predicting CV events in post-MI outpatients. The main
findings can be summarized as follows. First, the high levels of GDF-15 predicted a high incidence of CV events in this patient population; hs-CRP and ST2 levels did not. Second, we demonstrated that GDF-15 was an independent predictor of MACE in patients with prior MI, regardless of BNP levels, and a combined analysis of BNP and GDF-15 might improve risk stratification for CV events beyond clinical features.

Previous reports indicated that hs-CRP, a protein synthesized by the liver and widely used as an inflammatory biomarker, was strongly associated with CV disease morbidity and mortality. On the other hand, CRP is not a specific acute phase protein, has been shown to be a marker of systemic inflammation, and is elevated in response to infections and injuries. Therefore, hs-CRP may reflect inflammation from a variety of causes. GDF-15 is a distant member of the transforming growth factor-β superfamily. GDF-15 is not a cardiac-specific protein, and is upregulated in a variety of damaged tissues including the liver, kidney, lung, and heart. However, increased levels of GDF-15 have been observed in patients with AMI, heart failure, and cardiomyopathy. In the heart, GDF-15 levels increase in response to stress associated with tissue injury and inflammation, including myocardial ischemia. The induction of GDF-15 in cardiomyocytes because of myocardial ischemia in a mouse model of cardiac ischemia and reperfusion injury suggest that it could be a protective factor. ST2 is a member of the interleukin-1 receptor family with a soluble form that is markedly upregulated upon application of biomechanical strain to cardiac myocytes. Sabatine, et al demonstrated that high levels of ST2 at the onset of AMI were predictive of CV events at 30 days, and that the combination of ST2 and N-terminal prohormone BNP (NT-proBNP) significantly improved risk stratification for CV events.

In our study, high levels of GDF-15 predicted a high incidence of CV events in outpatients with prior MI, although hs-CRP and ST2 did not. This might be partially associated with the post-AMI expression patterns of these three inflammatory biomarkers. Mather, et al reported that serum levels of hs-CRP significantly decreased from one-week to one-month after AMI. ST2 concentrations are anticipated to increase on the first day after coronary occlusion and return to normal over the next 14 days. On the other hand, GDF-15 levels are elevated in patients with AMI, but do not show the typical rise and/or fall pattern seen with other markers of myonecrosis. Indeed, GDF-15 concentrations in patients with AMI remain stable for up to 72 hours. Bonaca, et al reported that GDF-15 concentrations did not significantly change from baseline (an average of 7 days after AMI) to 4 months after the event. In our study, the levels of hs-CRP and ST2 were relatively lower than values reported in the Framingham Heart Study. In that report, the median levels of hs-CRP, GDF-15, and ST2 in men were 1.81 mg/L, 1066 ng/L, and 23.6 ng/mL, respectively. Our study excluded patients with the presence of hemodynamic events (hypotension, congestive heart failure, and acute mitral regurgitation) and serious arrhythmic events in the post-AMI acute phase. These exclusion criteria might have contributed to the low levels of inflammatory biomarkers in the present study.

Khan, et al demonstrated that GDF-15 concentrations during the 3–5 days after AMI were independently related to age, impaired renal function, and BNP levels. In our study, GDF-15 concentrations one month after AMI were also related to the same factors, indicating that elevated levels of GDF-15 reflect a variety of clinical indicators of poor prognosis in the subject patient population. We demonstrated that the AUC of GDF-15 for predicting adverse events was the greatest among the inflammatory biomarkers examined (hs-CRP, GDF-15, and ST2). GDF-15 levels in stable outpatients were strong predictors of 3-year CV events, with an AUC of 0.75. The best cut-off value for MACE identification was 1221.0 ng/L, which was almost identical to the lower tertile value reported in a previous study. At this cut-off value, patients with higher GDF-15 levels had worse prognoses than those with lower biomarker levels. High GDF-15 levels were also independent predictors of MACE after multivariate analysis and provided a greater incremental predictive value compared with a model that included only age, renal function, and BNP level. Patients with high levels of BNP and GDF-15 also demonstrated a high risk of MACE. Therefore, a combined analysis of BNP and GDF-15 was more useful for risk stratification in post-MI outpatients than either biomarker alone. Although the mechanism of the association between GDF-15 and CV events might be multifocal, a previous report demonstrated that GDF-15 is associated with endothelial dysfunction and advanced coronary atherosclerosis, suggesting that GDF-15 levels may be related to chronic myocardial and vascular injury. In this study, GDF-15 showed a modest correlation with renal functions. The presence of chronic kidney disease is associated with CV events in patients with AMI. Thus, we need to pay attention to the use of GDF-15 as a prognosticator for CV events in patients with chronic kidney disease. Previous reports demonstrated that renal GDF-15 expression is upregulated in response to kidney injury, and urinary GDF-15 excretion becomes increased by proximal tubule injury. However, whether GDF-15 actively contributes to the development of chronic kidney disease due to its association with vascular injury or its direct effect on kidney remains to be investigated.

AMI survivors have a high risk of recurrent CV events,
such as sudden death and heart failure. However, the value of medical treatment for high GDF-15 levels in outpatients with prior MI is unknown; large-scale, randomized clinical trials addressing this issue have not been reported. A previous report demonstrated that GDF-15 concentrations did not significantly decline within 4 months after beginning statin therapy. Further studies are needed to determine whether active treatment to change GDF-15 levels, in the subject patient population, contributes to better prognoses.

Clinical implications: Our study demonstrated that BNP and GDF-15 measurements are useful for assessing the prognosis of clinically stable outpatients with prior MI, and clarified that GDF-15 provides incremental prognostic value over clinical features and BNP levels. We recommend that a combined analysis of BNP and GDF-15 levels should be performed in all post-MI outpatients to allow for precise risk stratification. The high adverse outcome rate in outpatients with high levels of GDF-15, independent of age, renal function, and BNP levels, necessitates early recognition and appropriate therapeutic intervention, such as optimal medical therapy and appropriate device therapy.

Study limitations: The major limitation of our study was the relatively small number of subjects. However, our study is the first to report on the utility of BNP and GDF-15 measurements in outpatients with prior MI, prospectively followed for a prolonged time. Second, we did not evaluate inflammatory biomarkers before the onset of AMI, and our analysis was based on single blood samples taken one-month post-AMI. Therefore, we could not clarify biomarker level changes during the follow-up period. Third, we analyzed the predictive value of biomarkers using individual cut-off concentrations, derived from an ROC curve analysis. Further studies are needed to determine the optimal biomarker cut-off points in this patient population. Despite these limitations, our findings provide new insight into the risk stratification for CV events in outpatients with prior MI.

Conclusions: High GDF-15 levels predicted a high incidence of CV events in stable outpatients with prior MI; hs-CRP and ST2 levels did not. GDF-15 provided an incremental prognostic value over clinical features and BNP levels in those patients.

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Disclosure

There are no conflicts of interest.

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