

# Low Plasma RANTES Levels Are an Independent Predictor of Cardiac Mortality in Patients Referred for Coronary Angiography

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**Objective**—Our objective was to evaluate the prognostic value of baseline plasma RANTES levels in patients with known or suspected coronary artery disease. RANTES is a chemokine produced by a variety of cell types including platelets that has been implicated in atherosclerosis.

**Methods and Results**—Baseline plasma RANTES levels were measured in 389 male patients undergoing coronary angiography at a Veterans Affairs Medical Center. The patients were followed-up prospectively for the occurrence of cardiac mortality and myocardial infarction. Follow-up data at 24 months were available for 97% of patients. In the entire cohort of patients, low baseline RANTES levels were an independent predictor of cardiac mortality. For cardiac death at 24 months, the survival rate was 87.3% in the lowest tertile of RANTES values, compared with 94% in the upper 2 tertiles combined ( $P=0.0298$  by log rank test). Furthermore, when patients were risk-stratified into those with and without an acute coronary syndrome, RANTES was an independent predictor of both cardiac mortality and myocardial infarction in those without an acute coronary syndrome. Finally, RANTES was also an independent predictor of cardiac mortality in the diabetic subset.

**Conclusions**—In a cohort of male patients undergoing coronary angiography, low baseline plasma RANTES levels are an independent predictor of cardiac mortality. (*Arterioscler Thromb Vasc Biol.* 2007;27:929-935.)

**Key Words:** atherosclerosis ■ chemokine ■ inflammation ■ prognosis ■ RANTES

Increasing evidence supports the involvement of inflammation in the initiation and progression of atherosclerosis.<sup>1,2</sup> Chemotactic cytokines or chemokines are small heparin-binding proteins that play a critical role in the inflammatory process by directing the migration of circulating leukocytes to sites of inflammation or injury.<sup>3</sup> They are classified into 4 major groups, according to the arrangement of the conserved cysteine (C) residues in the mature proteins.<sup>4</sup> CC chemokines, which have the first 2 conserved cysteine residues adjacent to each other, constitute the largest family of chemokines. They tend to attract mononuclear cells and are found at sites of chronic inflammation.<sup>3</sup>

CCL5 or RANTES (regulated on activation, normal T-cell expressed and secreted) is a CC chemokine that is stored in the alpha granules of platelets and that can be rapidly released after platelet activation.<sup>5</sup> After release from activated platelets, it can subsequently be deposited on inflamed or atherosclerotic endothelium and has been shown to mediate transmigration and shear-resistant arrest of monocytes onto activated endothelium.<sup>6</sup> In addition, RANTES is highly expressed within atheroma.<sup>7</sup> For these reasons, RANTES has been implicated in the genesis of atherosclerosis.<sup>8</sup> Despite

this, however, data regarding the significance of plasma RANTES levels in coronary artery disease (CAD) have been sparse and somewhat conflicting. On the one hand, RANTES levels in patients with acute coronary syndromes have been demonstrated to be elevated,<sup>9,10</sup> whereas levels in stable CAD have been shown to be downregulated.<sup>11</sup> In addition, gene polymorphisms that would be expected to result in reduced levels of RANTES have been shown to be associated with adverse cardiac events in a stable but high-risk population.<sup>12</sup> These observations raise the possibility that RANTES levels may have divergent implications in stable versus unstable CAD patients. To date, there have been no studies which have specifically looked at the prognostic value of baseline plasma RANTES levels in patients with known or suspected CAD. This is in contrast to other chemokines, such as IL-8 and MCP-1, which have also been associated with atherosclerosis and whose levels have been demonstrated to be prognostically significant.<sup>13,14</sup> Accordingly, and to this end, we sought to determine the prognostic value of baseline plasma RANTES levels in a broad population of patients referred for coronary angiography.

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## Methods

### Study Design

The study population and design have been previously described in detail elsewhere.<sup>15</sup> Briefly, 389 male patients undergoing coronary angiography for a variety of indications constituted the study population. Patients presenting with ST elevation myocardial infarction (MI), non-ST elevation MI, or unstable angina were classified as acute coronary syndrome (ACS). For the purposes of the present analysis, all other patients were classified as non-ACS, whether or not the principal indication for coronary angiography was related to chest pain. The primary endpoint of the study was cardiac mortality at 24 months for the entire cohort of 389 patients. The secondary endpoints of the study were MI at 24 months for the entire cohort, as well as cardiac mortality and MI for the ACS/non-ACS and the diabetic/nondiabetic subpopulations.

### Laboratory Methods

Aliquoted plasma samples stored at  $-70^{\circ}\text{C}$  were thawed and the levels of RANTES (R&D Systems, Minneapolis, Minn) and high-sensitivity C-reactive protein (hs-CRP) (Life Diagnostics, West Chester, Pa) were measured using commercially available enzyme-linked immunosorbent assay kits. The sensitivity of the RANTES and hs-CRP assays were 15.6 pg/mL and 0.1 mg/L, respectively. The intra-assay coefficients of variation for RANTES and hs-CRP were  $<5\%$  and  $<7.6\%$ , respectively. Fibrinogen was measured locally at the central laboratories of the Bronx VA Medical Center using the Beckman Coulter ACL Advance via the method of clot curve analysis. The white blood cell count was also measured locally in the same central laboratories.

### Statistical Methods

Patients were divided into tertiles according to their baseline RANTES values. Summary statistics for the continuous variables were presented both as means (with standard deviations) as well as medians (with interquartile ranges), and comparisons between the 3 groups were performed with the nonparametric Kruskal-Wallis test. All biomarkers were log transformed and normalized to reduce the skewness and kurtosis of the data. Categorical data were summarized as frequencies and percentages, and comparisons between the 3 groups were performed with Pearson  $\chi^2$  test or Fisher exact test.

The predictors of cardiac mortality and MI at 24 months were identified by univariable Cox regression. The results were presented as hazard ratios (HRs) and 95% confidence intervals (CIs). For time to occurrence of MI only the first MI was used in patients who experienced  $>1$  MI during the follow-up period. For each of the endpoints and for all of the patient groups analyzed, the following same baseline variables were studied by univariable analysis: age, family history of premature CAD, diabetes mellitus, hypertension, hyperlipidemia, active tobacco use, history of tobacco use, chronic renal insufficiency, body mass index, congestive heart failure on presentation, MI on presentation, aspirin use, beta-blocker use, angiotensin-converting enzyme inhibitor use, statin use, number of diseased coronary arteries, left ventricular function, fibrinogen, hs-CRP, white blood cell count, and RANTES. For the biomarkers the HRs represented an increase in one standard deviation in the respective log-transformed biomarker. RANTES was analyzed both as a continuous variable and as a categorical variable, in the latter case comparing the upper two tertiles of RANTES values to the lowest tertile. Only those univariable predictors with  $P<0.05$  were subsequently entered into multivariable Cox proportional hazards regression models. The independent predictors were identified using the backward elimination procedure.

Time-to-event at 24 months was presented with Kaplan-Meier curves for the individual endpoints of cardiac mortality and MI. Comparisons between the 2 groups defined as the upper 2 tertiles of RANTES values and the lowest tertile were performed with the log rank test.

All analyses used 2-sided tests with an overall significance level of  $\alpha=0.05$ .

## Results

### Baseline Characteristics and Association of RANTES With Clinical Variables

A total of 389 patients were enrolled in the study. The baseline characteristics of the study population stratified by tertiles of RANTES values are shown in Table 1. There were no significant associations between RANTES values and any of the other baseline clinical, laboratory, or angiographic variables.

The breakdown of the patient population based on the primary indication for coronary angiography was as follows: ST-segment elevation MI (STEMI), 23 patients (5.9%); non-ST-segment elevation MI (NSTEMI), 84 patients (21.6%); troponin-negative unstable angina, 86 patients (22.1%); stable angina pectoris, 155 patients (39.8%); evaluation of congestive heart failure and/or left ventricular dysfunction, 24 patients (6.2%); and evaluation of valvular heart disease, 17 patients (4.4%). Using the definition of ACS described in the Methods section, 193 patients (49.6%) were further classified as having ACS, whereas 196 patients (50.4%) were classified as not having ACS.

### Association Between Baseline Plasma RANTES Levels and Clinical Outcomes at 24 Months for the Entire Sample

Two-year follow-up data were available for 97% of the patients. In the entire population, there were a total of 51 deaths (13%), of which 31 (61%) were classified as cardiac in etiology. Similarly, MI developed in 61 (16%) by 24 months, of which 10 MIs were fatal.

Using the lowest tertile of RANTES values as a prespecified cut-off point (ie,  $<1949.80$  and  $\geq 1949.80$  pg/mL), Kaplan-Meier curves were derived for the entire cohort of patients (Figure 1). Kaplan-Meier plots demonstrated a significant increase in cardiac mortality for patients with RANTES values in the lowest tertile (ie,  $<1949.80$  pg/mL). For cardiac death at 24 months, the survival rate was 87.3% for the lowest tertile, compared with 94% for the upper two tertiles combined ( $P=0.0298$  by log rank test).

Together with RANTES values, all baseline variables shown that were significant for their association with cardiac mortality on univariable analysis were entered into a backward elimination multivariable Cox regression model. After adjusting for these variables, RANTES (analyzed as a continuous variable) was found to be an independent predictor of cardiac mortality (Table 2) (HR, 0.64; 95% CI, 0.45 to 0.92;  $P=0.0165$ ). The other independent predictors of cardiac mortality were the number of diseased coronary arteries, congestive heart failure on presentation, and chronic renal insufficiency.

RANTES was also analyzed as a categorical variable, comparing the upper 2 tertiles of RANTES values to the lowest tertile (Table 2). RANTES levels were again a strong and independent predictor of cardiac mortality (HR, 0.42; 95% CI, 0.20 to 0.88;  $P=0.0213$ ) for the combined upper 2 tertiles of RANTES values compared with the lowest tertile.

RANTES levels were not a predictor of MI in the total population of patients.

TABLE 1. Baseline Characteristics of the Entire Population Stratified by Tertiles of Baseline Plasma RANTES Values

| Characteristics                    | Statistics                                    | RANTES <1949.80 pg/mL | RANTES ≥1949.80 and <5644.78 pg/mL | RANTES ≥5644.78 pg/mL | P      |
|------------------------------------|-----------------------------------------------|-----------------------|------------------------------------|-----------------------|--------|
|                                    |                                               | n=126*                | n=125*                             | n=126*                |        |
| Age                                | Mean (std)                                    | 65.2 (10.3)           | 65.9 (10.3)                        | 64.5 (9.3)            | 0.4273 |
|                                    | Median                                        | 67.3                  | 67.3                               | 64.6                  |        |
|                                    | (25 <sup>th</sup> , 75 <sup>th</sup> )        | 55.6, 73.1            | 58.0, 74.2                         | 57.0, 73.4            |        |
| Race                               | Black n (%)                                   | 39 (31.0)             | 40 (32.0)                          | 42 (33.3)             | 0.9638 |
|                                    | Hispanic n (%)                                | 39 (31.0)             | 34 (27.2)                          | 35 (27.8)             |        |
|                                    | White n (%)                                   | 48 (38.1)             | 51 (40.8)                          | 49 (38.9)             |        |
| Family history of premature CAD    | n (%)                                         | 32 (25.4)             | 35 (28.0)                          | 26 (20.6)             | 0.3895 |
| Diabetes mellitus                  | n (%)                                         | 53 (42.1)             | 55 (44.0)                          | 55 (43.7)             | 0.9469 |
| Hypertension                       | n (%)                                         | 101 (80.2)            | 104 (83.2)                         | 109 (86.5)            | 0.4014 |
| History of tobacco use             | n (%)                                         | 101 (80.2)            | 103 (82.4)                         | 105 (83.3)            | 0.7971 |
| Active tobacco use                 | n (%)                                         | 40 (31.8)             | 37 (29.6)                          | 42 (33.3)             | 0.8156 |
| Hyperlipidemia                     | n (%)                                         | 68 (54.0)             | 72 (57.6)                          | 65 (51.6)             | 0.6290 |
| Chronic renal insufficiency        | n (%)                                         | 5 (4.0)               | 7 (5.6)                            | 6 (4.8)               | 0.8321 |
| Fibrinogen                         | Mean (SD)                                     | 381.4 (106)           | 372.4 (114.7)                      | 367.9 (111.7)         | 0.2398 |
|                                    | Median (25 <sup>th</sup> , 75 <sup>th</sup> ) | 366.5 (316.5, 425)    | 359.5 (286.5, 433)                 | 344.0 (293, 427)      |        |
| WBC                                | Mean (SD)                                     | 7.50 (2.3)            | 7.60 (2.5)                         | 7.4 (2.3)             | 0.8531 |
|                                    | Median (25 <sup>th</sup> , 75 <sup>th</sup> ) | 7.1 (5.9, 8.6)        | 7.2 (5.9, 8.8)                     | 7.2 (5.8, 8.4)        |        |
| hs-CRP                             | Mean (SD)                                     | 21.9 (37.8)           | 25.9 (45.6)                        | 23.4 (37.5)           | 0.4220 |
|                                    | Median (25 <sup>th</sup> , 75 <sup>th</sup> ) | 8.4 (3.2, 17.1)       | 8.7 (3.8, 18.8)                    | 10.8 (4.4, 21.2)      |        |
| BMI                                | Mean (SD)                                     | 29.4 (6.6)            | 28.2 (4.9)                         | 28.4 (5.4)            | 0.4503 |
|                                    | Median (25 <sup>th</sup> , 75 <sup>th</sup> ) | 28.2 (25.5, 32.9)     | 27.9 (25.1, 30.6)                  | 27.6 (24.2, 31.2)     |        |
| CHF on presentation                | n (%)                                         | 34 (27.0)             | 36 (28.8)                          | 27 (21.4)             | 0.3790 |
| MI on presentation                 | n (%)                                         | 37 (29.4)             | 27 (21.6)                          | 40 (31.8)             | 0.1709 |
| ASA use                            | n (%)                                         | 107 (84.9)            | 105 (84.0)                         | 108 (85.7)            | 0.9306 |
| Beta-blocker use                   | n (%)                                         | 82 (65.1)             | 81 (64.8)                          | 95 (75.4)             | 0.1195 |
| ACE inhibitor use                  | n (%)                                         | 80 (63.5)             | 81 (64.8)                          | 67 (53.2)             | 0.1184 |
| Statin use                         | n (%)                                         | 64 (50.8)             | 68 (54.4)                          | 65 (51.6)             | 0.8349 |
| No. of diseased coronary arteries† | 0                                             | 28 (22.2)             | 22 (17.6)                          | 21 (16.7)             | 0.4824 |
|                                    | ≥1                                            | 98 (77.8)             | 103 (82.4)                         | 105 (83.3)            |        |
| LV function                        | EF≥55%                                        | 41 (34.8)             | 44 (37.6)                          | 46 (38.3)             | 0.4909 |
|                                    | EF 45–54%                                     | 24 (20.3)             | 28 (23.9)                          | 34 (28.3)             |        |
|                                    | EF 31–44%                                     | 36 (30.5)             | 26 (22.2)                          | 23 (19.2)             |        |
|                                    | EF≤30%                                        | 17 (14.4)             | 19 (16.2)                          | 17 (14.2)             |        |
| Clinical syndrome                  | ACS n (%)                                     | 59 (46.8)             | 59 (47.2)                          | 68 (54.0)             | 0.4432 |
|                                    | Non-ACS n (%)                                 | 67 (53.2)             | 66 (52.8)                          | 58 (46.0)             |        |

\*Because of 12 missing values for RANTES, total n=377.

†Takes into account the left main, left anterior descending, left circumflex, and right coronary arteries (minimum=0, maximum=4).

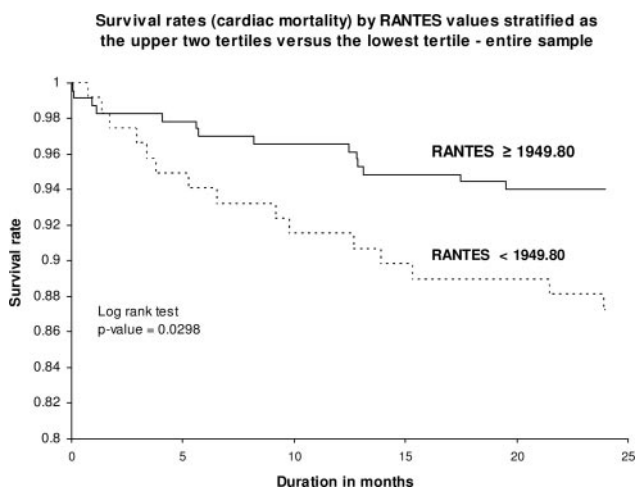
ACE indicates angiotensin-converting enzyme; ASA, aspirin; BMI, body mass index; CAD, coronary artery disease; CHF, congestive heart failure; hs-CRP, high-sensitivity C-reactive protein; LV, left ventricular; MI, myocardial infarction; WBC, white blood cell count.

### Clinical Outcomes for the ACS and Non-ACS Samples

Although not reaching statistical significance, patients with ACS had a numerically higher median RANTES level than their non-ACS counterparts (3229 versus 2909 pg/mL, respectively;  $P=0.4084$ ). Given this observation, and in an attempt to determine if the predictive power of RANTES was either related to or affected by baseline risk, additional

analyses were performed in each of these 2 subpopulations (ie, ACS and non-ACS).

As was performed for the entire cohort, Kaplan-Meier curves were derived for the non-ACS subpopulation of patients using the lowest tertile of RANTES values as a prespecified cut-off point (Figure 2). Kaplan-Meier plots demonstrated a significant increase in cardiac mortality for patients in the lowest tertile of RANTES values. For cardiac



**Figure 1.** Kaplan-Meier curves for cardiac mortality according to baseline plasma RANTES values comparing the upper 2 tertiles to the lowest tertile in the entire sample of patients. At 24 months, the number of events for the lowest tertile was 15 (12.7%), whereas the number of events for the combined upper 2 tertiles was 14 (6.0%).

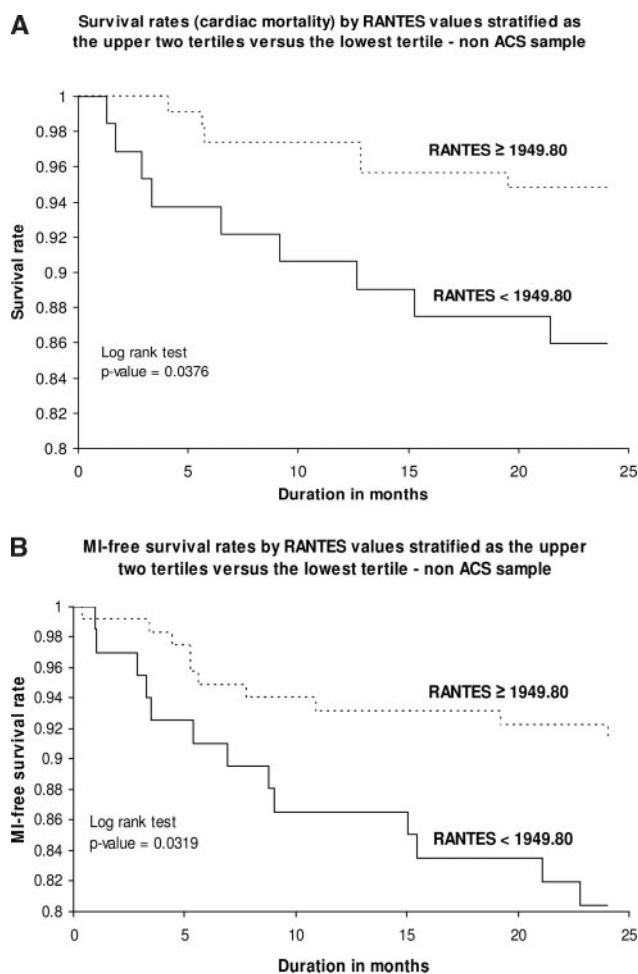
death at 24 months, the survival rate was 85.9% in the lowest tertile, compared with 94.8% for the upper 2 tertiles combined ( $P=0.0376$  by log rank test). Similarly, Kaplan-Meier plots also demonstrated a significant decrease in MI-free survival for patients in the lowest tertile compared with those in the upper 2 tertiles combined (80.6% versus 91.5%, respectively;  $P=0.0319$  by log rank test).

To determine if RANTES was an independent predictor of cardiovascular outcomes in the non-ACS subpopulation, multivariable Cox regression analyses were performed in the same manner as described for the entire cohort. For the non-ACS subpopulation, RANTES (analyzed as a continuous variable) was of borderline significance for predicting cardiac mortality (unadjusted HR, 0.63; 95% CI, 0.39 to 1.00;  $P=0.0506$ ) and not significant for predicting MI (unadjusted HR 0.71, 95% CI 0.48 to 1.04;  $P=0.0766$ ). However, when analyzed as a categorical variable and after adjusting for other significant baseline univariable predictors of both cardiac mortality and MI (in separate models), RANTES was found to be an independent predictor of each of these individual

**TABLE 2. Multivariable Cox Proportional Hazards Analyses for Cardiac Mortality in the Entire Sample**

| Variable                          | P      | HR and 95% CI     |
|-----------------------------------|--------|-------------------|
| <b>Model A</b>                    |        |                   |
| RANTES (continuous)*              | 0.0165 | 0.64 (0.45, 0.92) |
| No. of diseased coronary arteries | 0.0021 | 1.84 (1.25, 2.71) |
| CHF on presentation               | 0.0038 | 3.05 (1.44, 6.48) |
| Chronic renal insufficiency       | 0.0436 | 2.84 (1.03, 7.85) |
| <b>Model B</b>                    |        |                   |
| RANTES (categorical)              | 0.0213 | 0.42 (0.20, 0.88) |
| No. of diseased coronary arteries | 0.0005 | 1.94 (1.34, 2.80) |
| CHF on presentation               | 0.0014 | 3.28 (1.58, 6.80) |

\*RANTES was analyzed as a continuous variable with hazards ratio (HR) per 1 SD increment of log-transformed RANTES, where 1 SD=1.06.



**Figure 2.** Kaplan-Meier curves for (A) cardiac mortality and (B) MI-free survival (time-to-first MI) according to baseline plasma RANTES values stratified by the lowest tertile in the non-ACS sample of patients. At 24 months, the number of events for the lowest tertile versus the combined upper two tertiles for (A) cardiac mortality were 9 (14.1%) and 5 (5.2%), respectively. The number of events for the lowest tertile versus the combined upper 2 tertiles for (B) first MI were 13 (19.4%) and 10 (8.5%), respectively.

outcomes in the non-ACS subpopulation (Table 3). Compared with the lowest tertile of RANTES values, the HRs for the combined upper two tertiles for cardiac mortality and MI were 0.24 (95% CI, 0.07 to 0.78;  $P=0.0176$ ) and 0.41 (95% CI, 0.18 to 0.93;  $P=0.0339$ ), respectively. RANTES was not a predictor of either outcome in the ACS subpopulation. In the ACS group, the independent predictors of cardiac mortality at 24 months were age and hs-CRP, whereas the only independent predictor of MI was the number of diseased coronary arteries.

### Clinical Outcomes for the Diabetic Sample

Of the total population of 389 patients, 169 (43%) had diabetes. Given the known association of diabetes with atherosclerosis, additional analyses were performed in the diabetic and non-diabetic subpopulations.

Using the same cut-off values for RANTES described, Kaplan-Meier curves were derived for the diabetic subset (Figure 3). These plots demonstrated a significant increase in

**TABLE 3. Multivariable Cox Proportional Hazards Analyses for Cardiac Mortality and MI in the Non-ACS Sample**

| Variable                          | P      | HR and 95% CI      |
|-----------------------------------|--------|--------------------|
| <b>Cardiac Mortality</b>          |        |                    |
| RANTES (categorical)              | 0.0176 | 0.24 (0.07, 0.78)  |
| No. of diseased coronary arteries | 0.0012 | 2.36 (1.41, 3.96)  |
| CHF on presentation               | 0.0024 | 5.10 (1.78, 14.59) |
| Chronic renal insufficiency       | 0.0240 | 7.26 (1.30, 40.56) |
| <b>Myocardial Infarction</b>      |        |                    |
| RANTES (categorical)              | 0.0339 | 0.41 (0.18, 0.93)  |
| No. of diseased coronary arteries | 0.0002 | 2.03 (1.40, 2.97)  |
| CHF on presentation               | 0.0024 | 3.63 (1.58, 8.35)  |

ACS indicates acute coronary syndrome.

cardiac mortality for patients with RANTES values in the lowest tertile. For cardiac death at 24 months, the survival rate was 80% in the lowest tertile, compared with 93.4% for the upper 2 tertiles combined ( $P=0.0126$  by log rank test).

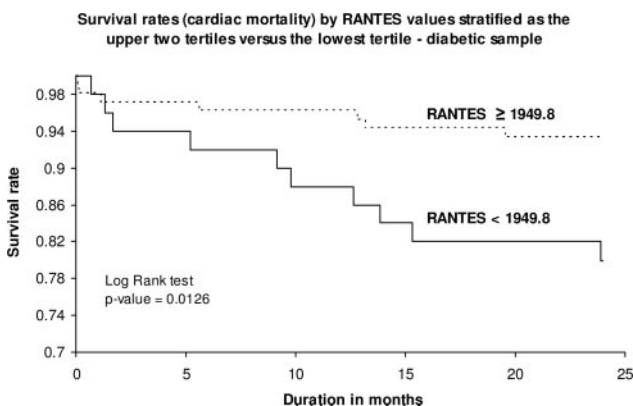
To determine if RANTES was an independent predictor of cardiovascular outcomes in the diabetic subset, RANTES was again analyzed using multivariable Cox regression models. When analyzed as a continuous variable, RANTES was of borderline significance for predicting cardiac mortality (unadjusted HR, 0.67; 95% CI, 0.44 to 1.02;  $P=0.0595$ ). However, when analyzed as a categorical variable and after adjusting for baseline variables that were significant on univariable analysis for their association with cardiac mortality, RANTES was found to be an independent predictor of this outcome in the diabetic subpopulation. The HR was 0.31 (95% CI, 0.12 to 0.83;  $P=0.0191$ ) for the combined upper 2 tertiles of RANTES values compared with the lowest tertile. RANTES was not a predictor of MI in the diabetic subpopulation. In the nondiabetic group, RANTES was not a predictor of any cardiovascular outcome.

## Discussion

Increasing evidence implicates inflammation in the initiation and progression of atherosclerosis.<sup>1,2</sup> Chemotactic cytokines,

or chemokines, are a family of small secreted proteins that are critical to the inflammatory process by virtue of their ability to direct the migration of leukocytes to sites of vascular injury and inflammation, including developing atherosclerosis.<sup>3,8,16</sup> Chemokines induce cell migration and activation by binding specific G-protein–coupled cell-surface receptors on target cells.<sup>4</sup> The cell type-specific expression of these receptors appears to be the major determinant of the leukocyte specificity of chemokine action.<sup>4,17</sup>

The chemokine CCL5/RANTES is a soluble chemokine secreted by many different cell types, such as endothelial cells, smooth muscle cells, activated T cells, macrophages, and platelets.<sup>5,7,18,19</sup> Platelets sequester RANTES protein in their  $\alpha$ -granules and release it during acute stages of inflammation.<sup>6,17,20</sup> RANTES is a potent chemoattractant for T cells, monocytes, natural killer cells, basophils, and eosinophils.<sup>5,17,21</sup> It is a ligand for a number of chemokine receptors including, CCR1, CCR3, CCR5, CCR9, and DARC (Duffy antigen receptor for chemokines) in humans.<sup>17</sup> Chemokines such as RANTES are thought to play pivotal roles in the cellular infiltrates that underlie various disease processes, and have been implicated in atherosclerosis.<sup>7,8,22,23</sup> In the present study, we demonstrate for the first time that baseline plasma RANTES levels are powerful and independent predictors of cardiac mortality in a broad population of patients referred for coronary angiography. Furthermore, in the subset of such patients who do not have an ACS, baseline RANTES levels are in addition independently predictive of MI. An additional group in whom low RANTES levels appear to be strongly predictive of events is the diabetic subset, possibly reflecting the greater atherosclerotic burden known to be present in this population. While the finding that low, rather than high, levels of RANTES are associated with adverse outcomes may at first seem counterintuitive, we believe that our data are in accord with the published literature in this regard. Rothenbacher et al recently investigated the association of several chemokines with the risk of stable coronary heart disease in a large case-control study and found that serum levels of RANTES were lower in coronary heart disease patients compared with age- and gender-matched controls, even after adjustment for conventional coronary heart disease risk factors such as diabetes mellitus.<sup>11</sup> This was in contradistinction to other chemokines, such as IP-10 and IL-8, whose levels were increased in patients with coronary heart disease. Even more germane to our findings with respect to both prognosis and diabetes, Boger et al studied the prognostic importance of functional polymorphisms of several chemokines and their receptors in 225 stable but high-risk patients with diabetes mellitus and end-stage renal disease (ESRD) on dialysis.<sup>12</sup> They found that patients carrying the RANTES –403A and In1.1C alleles had a significantly higher risk for cardiac mortality on multivariable analysis. Although plasma or serum levels of RANTES were not measured in that study, it is noteworthy that patients carrying these alleles would be expected to be low expressers of RANTES.<sup>12,24</sup> Equally important, all patients in the study by Boger et al had diabetes, further underscoring our findings of the prognostic significance of low RANTES levels in the diabetic population.



**Figure 3.** Kaplan-Meier curves for cardiac mortality according to baseline plasma RANTES values stratified by the lowest tertile in the diabetic sample of patients. At 24 months, the number of events (cardiac deaths) for the lowest tertile versus the combined upper 2 tertiles were 10 (20%) and 7 (6.6%), respectively.

While the precise mechanism by which low levels of RANTES are associated with CAD and adverse events remains to be elucidated, there are a number of explanations based on the known physiology of RANTES and its receptors. One potential mechanism may relate to the upregulation of the CCR5 receptor caused by low RANTES levels. The CCR5 receptor is well-known to mediate the transmigration of leukocytes on inflamed endothelium, and has been recently identified as the crucial RANTES receptor associated with atherosclerosis.<sup>12,22,25–27</sup> Indeed, upregulation of CCR5 in patients with polymorphisms that would be expected to lead to low RANTES expression has been proposed as a mechanism by which low RANTES could be associated with adverse cardiovascular outcomes.<sup>12</sup> Alternatively, the inverse association between RANTES levels and atherosclerosis may instead reflect increased deposition of RANTES on the vascular endothelium leading to greater CCR5 stimulation. It is known that platelet microparticles contain substantial amounts of RANTES and deposit RANTES on activated endothelium or atherosclerotic arteries.<sup>28</sup> Thus, it is conceivable that the pool of RANTES measurable by routine enzyme-linked immunosorbent assay could be diminished in patients with atherosclerosis who would be expected to have more extensive deposition of RANTES on activated endothelium. Indeed, von Hundelshausen required the use of a modified enzyme-linked immunosorbent assay (with perfusion with platelets or exposure to their supernatants) to detect the binding of endothelial surface adherent RANTES.<sup>6</sup>

The finding that RANTES levels were predictive of cardiac mortality and MI in the stable non-ACS population but not in the ACS population is intriguing. RANTES levels have been shown to be elevated acutely in patients with an ACS<sup>9,10</sup> and, as already stated, this is in contrast to the low levels seen in the setting of chronic and stable CAD.<sup>11</sup> Although not reaching statistical significance, ACS patients in our study did have numerically higher RANTES levels than their non-ACS counterparts. The known elevation of RANTES levels in patients with ACS is believed to result from the acute release of RANTES from activated platelets known to be present in patients with unstable angina and acute myocardial infarction.<sup>9</sup> Activated platelets have been characterized as having a spontaneous and markedly enhanced release of RANTES.<sup>29</sup> We believe that the inability of RANTES levels to predict long-term adverse cardiovascular outcomes in ACS may be related to the acute spike in RANTES levels that occur in this particular setting. Such a release may be akin to the transient elevation of hs-CRP or other acute phase reactants in patients with an acute inflammatory disorder. Thus, just as acute nonspecific elevations of hs-CRP may hinder the ability to use hs-CRP levels for cardiac prognostic purposes, it is conceivable that elevations of RANTES levels caused by acutely activated platelets in the ACS setting may obscure the predictive value of low RANTES levels in chronic and stable CAD.

This study has a number of limitations. First, it was conducted in an exclusively male population. Second, the population was a high-risk cohort as evidenced by both clinical and laboratory parameters as well by the high event rate for death and MI. Therefore, it is unknown whether

RANTES levels would be similarly predictive of events in a lower-risk population. Along the same lines, larger studies in healthy populations are needed to determine the reference range of RANTES and the factors that influence its levels in the absence of atherosclerotic disease. Third, the size of the population was small and the study was not designed with a priori calculations with respect to sample size or statistical power. As such, the findings need to be confirmed in larger and prospectively designed studies.

In conclusion, we found that low baseline plasma RANTES levels are independently associated with an increased risk of cardiac death at 2-year follow-up in an unselected population of males referred for coronary angiography. The prognostic power of RANTES in this regard is even stronger in the non-ACS subpopulation of patients, in which baseline levels are independently predictive of both cardiac mortality and MI. Importantly, this study is consistent with the recent observations that low, rather than high, RANTES levels are associated with the presence of established CAD.

## Disclosures

None.

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