Fetuin-A Is an Independent Predictor of Death after ST-Elevation Myocardial Infarction

PASCAL LIM,1* JEAN-PHILLIPE COLLET,2† STEPHANE MOUTEREAU,3‡ NATHALIE GUIGUI,1 LAURENS MITCHELL-HEGGS,1 SYLVAIN LORIC,3 MAGY BERNARD,4 SAID BENHAMED,4 GILLES MONTALESCOT,2 JEAN-LUC DUBOIS RANDÉ,1 and PASCAL GUERET1

Background: Fetuin-A inhibits inflammation and has a protective effect against myocardial ischemia. Its deficiency has been found to be associated with cardiovascular death in patients with end-stage renal failure disease. We investigated the association between plasma fetuin-A and clinical outcome after ST-elevation acute myocardial infarction (STEMI).

Methods: We measured fetuin-A in 284 consecutive patients with STEMI and correlated these data with the occurrence of death at 6 months (n = 25). We also measured fetuin-A in a control group and chose the 95th percentile as the cutoff to define abnormality.

Results: Patient mean (SD) age was 60 (14) years, and creatinine clearance was 83 (31) mL/min; 82% were men. Mean (SD) plasma fetuin-A concentrations at admission [188 (69) mg/L, P = 0.01] and at day 3 [163 (57) mg/L, P < 0.0001] were lower in patients than in controls [219 (39) mg/L; 95th percentile 140 mg/L]. Fetuin-A <140 mg/L was observed in 20% of patients at admission vs 40% at day 3 (P < 0.001). Fetuin-A concentrations did not correlate with peak cardiac troponin values but did correlate inversely with C-reactive protein (CRP) and NT-pro-brain natriuretic peptide (NT-proBNP). Fetuin-A <140 mg/L at admission (OR = 3.3, P = 0.03) and at day 3 (OR = 6.3, P = 0.002) was an independent correlate of death at 6 months, irrespective of NT-proBNP, CRP, or Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications (CADILLAC) risk score. Conversely, fetuin-A ≥140 mg/L was associated with an excellent survival rate [negative predictive value (NPV) = 97% overall], even in high-risk populations with CADILLAC risk score ≥6 (NPV = 90% in patients).

Conclusions: Fetuin-A is an important predictor of death at 6 months in STEMI patients independent of NT-proBNP, CRP, and CADILLAC risk score.

Atherosclerosis is a chronic inflammatory disease of the arterial wall (1) characterized by a progressive accumulation of lipids, cells (macrophages, T lymphocytes, and smooth muscle cells), and extracellular matrix. Inflammation is involved throughout the different stages of atherosclerosis and is a strong determinant of plaque disruption and thrombosis (2). This process is naturally counteracted by antiinflammatory cytokines such as interleukin (IL)-10, an inhibitor of proinflammatory transcription factor NF-κB nuclear localization (3), leading to suppressed cytokine production (4). Furthermore, accumulating evidence suggests that inflammatory balance (5, 6) and especially antiinflammatory factors are determinant for the prognosis of atherosclerotic disease (7, 8). Fetuin-A/a2-Heremans–Schmid glycoprotein is an antiinflammatory mediator that is produced by the liver and participates in macrophage deactivation (9–11). Fetuin-A enhances the

1 Department of Cardiology, Assistance Publique Hôpitaux de Paris, Henri Mondor Hospital, Créteil, France.
2 Department of Cardiology, Assistance Publique Hôpitaux de Paris, Institut National de la Santé et de la Recherche Médicale 856, Pitie Salpetriere Hospital, Paris, France.
3 Biochemistry Laboratory, Assistance Publique Hôpitaux de Paris, Henri Mondor Hospital, Créteil, France.
4 Biochemistry Laboratory, Assistance Publique Hôpitaux de Paris, Institut National de la Santé et de la Recherche Médicale 856, Pitie Salpetriere Hospital, Paris, France.
† Address correspondence to this author at: Henri Mondor Hospital, Department of Cardiology, 51 Av du Maréchal de Lattre de Tassigny, 94010 Créteil, France. Fax 33-1-49812801; e-mail lim.pascal.hmn@gmail.com.
‡ These authors contributed equally to this study.
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Nonstandard abbreviations: IL, interleukin; ESRD, end-stage renal disease; STEMI, ST-elevation acute myocardial infarction; CADILLAC, Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications; OR, odds ratio; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention; LLD, lowest level of detection; CRP, C-reactive protein; NT-proBNP, NT-pro-brain natriuretic peptide; cTnl, troponin Ic; AUC, area under the curve; ACS, acute coronary syndrome.
cellular uptake of cationic inhibitors of proinflammatory cytokine synthesis and thus prevents the self-amplification of inflammatory response. Fetuin-A also inhibits insulin receptor autophosphorylation and tyrosine kinase activity (12). Recent studies have suggested that fetuin-A exerts a protective effect against ischemia in the cardiomyocyte (13, 14). Fetuin-A also regulates bone mineralization processes by preventing ectopic calcification (15–18). In patients undergoing dialysis, low plasma fetuin-A is associated with cardiovascular death (19, 20). However, the prognostic value of fetuin-A has not been evaluated in patients with atherothrombosis and without end-stage renal disease (ESRD). To address this issue, we measured the fetuin-A plasma concentration in ST-elevation acute myocardial infarction (STEMI) patients and correlated it with outcome at 6 months.

**Materials and Methods**

The study included 284 consecutive patients admitted for STEMI with completed 6-month follow-up. All baseline characteristics were recorded (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol53/issue10) and the Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications (CADILLAC) risk score was calculated as described (21). Briefly, we computed the score from 7 independent variables weighted to their respective odds ratio (OR) [age >65 years (2 points), admission heart failure (3 points), baseline left ventricular ejection fraction (LVEF) <40% (4 points), anemia (2 points, defined as a baseline hematocrit <39% for men and <36% for women), creatinine clearance <60 mL/min (3 points), triple-vessel disease (2 points), and postprocedural TIMI (Thrombolysis in Myocardial Infarction) flow grade <III (2 points)]. One-year mortality after STEMI was low (<1%) for a score ≤2, intermediate (4%–5%) between 3 and 5, and high (>12%) for ≥6. STEMI was defined by a continuous and persistent typical chest pain (>30 min duration) and ST-segment elevation >2 mm in >2 contiguous precordial leads or 1 mm in >2 contiguous limb leads. All revascularized patients were treated by percutaneous coronary intervention (PCI). Dialysis patients and those with ESRD (creatinine clearance <15 mL/min) presenting with any active infection, chronic inflammatory disease, or known malignancy process were excluded. LVEF was determined either by 2-dimensional echocardiography (Simpson method) or by angiography during hospitalization. The study was approved by our institutional review committee, and informed consent was obtained from all participants.

For all patients, 6-month follow-up was obtained by direct patient interview or from the referring physician or relatives. The primary endpoint was death from any cause. Cardiovascular death was defined as sudden unexplained death and death from heart failure or refractory ventricular arrhythmias.

We collected blood samples at admission and on day 3. They were centrifuged immediately, and the serum was separated and stored at −80 °C for future analysis. We measured serum fetuin-A concentrations by use of a semiautomated sandwich immunoenzymometric assay (EDI™ Human Fetuin-A ELISA, Epitope Diagnostics). Briefly, a purified human fetuin-A–specific polyclonal antibody was used as the capture antibody, and another purified human fetuin-A–specific antibody from a different animal species (labeled) was used as the detection antibody. The 1st incubation time, substrate incubation time, and antibody tracer incubation time were 2 h, 30 min, and 20 min, respectively. Reagent distribution and predilution of samples were performed using an automated workstation (Freedom Evo, Tecan). The final dilution reached after a cascade of 3 successive dilutions was 1/4096. The lowest level of detection (LLD) for human fetuin-A was 0.025 g/L and was calculated as the mean + 3 SD of 10 successive measurements of assay buffer samples. The linear measurement ranged up to 7 g/L according to the manufacturer’s specifications. Variability was assessed on 4 different series, and the result was close to the manufacturer’s specifications. Intraassay variation was <5.5%, and interassay variation <6.8%, determined on 9 different samples tested, with concentrations ranging from 0.110 to 0.260 g/L. Control fetuin-A concentrations were determined in 34 individuals [20 men, mean (SD) age 67 (14) years, creatinine clearance 85 (33) mL/min] without history of inflammatory disease, heart failure, coronary artery disease, or altered LVEF. These men were consecutively enrolled among outpatients examined in the echocardiography laboratory. The fetuin-A concentration was 219 (39) mg/L (95% CI 140–297), and the percentile cutoff (<mean − 2 SD) was chosen to define abnormality (<140 mg/L). We measured C-reactive protein (CRP) concentrations by use of an immunoturbidimetry method (Bayer) in all patients, and when CRP was <2 mg/L, high-sensitivity CRP by immunonephelometry (Dade Behring) was also performed. LLD and total imprecision were 0.5 mg/L and 4.6% at 4 mg/L, respectively, for CRP and 0.1 mg/L and <5% at 0.5 mg/L, respectively, for high-sensitivity CRP. NT-pro-brain natriuretic peptide (NT-proBNP) was measured using an NT-proBNP Roche Diagnostics assay on an Elecsys 2010 analyzer. The NT-proBNP upper reference limit was 120 ng/L, determined as the 97.5th percentiles of NT-proBNP value in healthy blood donors (153 ng/L for females and 88 ng/L for males), and the total imprecision at 355 ng/L was 2.9%. We assessed troponin Ic (cTnI; Dade Behring) every 6 h during the 1st 24 h to determine the peak value. LLD and total imprecision were 0.04 μg/L and 7.5% at 0.52 μg/L, respectively, for cTnI. The 99th percentile of our control population was 0.08 μg/L, and the cutoff used was < 0.1 μg/L. Creatinine clearance was calculated using the standard Cockcroft–Gault formula (22, 23).

The sample size was not statistically calculated when the study was designed because of the lack of published
data on the relationship between fetuin-A and death after STEMI. Gaussian-distributed continuous variables (Wilk-Shapiro test) were expressed as mean (SD), and skewed variables as median and interquartile range (25th and 75th percentiles). For variables that were not gaussian distributed we used logarithm-transformed values for Pearson or logistic regression analysis and presented dichotomous data as percentages. To compare numerical data between 2 or several groups, we used ANOVA and Mann–Whitney and Kruskal–Wallis tests when appropriate. We assessed dichotomized comparison by $\chi^2$ test or Fisher exact test. To identify independent predictors of death, variables with $P < 0.1$ were entered in the multivariate logistic model using a stepwise method. Two models were tested, the 1st including only admission biomarker values, and the 2nd only day 3 values. For the 2 models, CADILLAC risk score and fetuin-A were included as continuous variables. We repeated the multivariable analysis using the fetuin-A cutoff value (<140 mg/L) derived from the control population to display the impact of low fetuin-A on mortality. ROC curves were computed for significant predictors to determine their accuracy and optimal cutoff to predict death. We considered 2-tailed $P$ values $<0.05$ statistically significant. Analysis was performed using SPSS 11.0 for Windows.

### Results

The study population consisted of 284 consecutive patients [60 (14) years, 82% male, 82% white, mean creatinine clearance 83 (31) mL/min (range 15–183)] admitted for STEMI. Prehospital fibrinolysis therapy was performed in 14% of the patients (40 of 284), and 97% (n = 275 of 284) underwent immediate and systematic PCI. Glycoprotein IIb/IIIa inhibitors were administered in 90% (n = 255 of 284) of patients as an adjunctive therapy to primary PCI. Baseline population characteristics are summarized in Table 1 in the online Data Supplement.

Fetuin-A was lower at admission [188 (69) mg/L, $P = 0.01$] and at day 3 [163 (57) mg/L, $P < 0.0001$] in patients than in the control group [219 (39) mg/L]. We observed low fetuin-A concentrations (<140 mg/L) in 20% at admission vs 40% at day 3 ($P < 0.001$). Population characteristics significantly associated with a low fetuin-A concentration are reported in Table 1. Overall, admission and day 3 fetuin-A concentrations did not correlate with current cardiovascular risk factors, peak troponin, or time interval between the onset of symptoms and reperfusion therapy. A low fetuin-A concentration was associated with older patients, admission heart failure, higher NT-proBNP and CRP concentrations, and lower LVEF and creatinine clearance.

At 6 months, 25 patients had died, including 11 sudden deaths or refractory arrhythmias, 12 acute refractory heart failure episodes, 1 free wall rupture, and 1 fatal bleed. By univariate analysis (see Table 1 in the online Data Supplement), low fetuin-A concentration, high CRP and NT-proBNP, CADILLAC risk score, and peak troponin were significantly associated with death, whereas there was a trend toward statistical significance for diabetes ($P = 0.06$) and delay from symptom onset to revascularization ($P = 0.08$). After multivariable analysis (Table 2), independent correlates of death were CADILLAC risk score and low fetuin-A and high NT-proBNP concentrations at admission and day 3.

Prediction of death with day 3 values was more accurate than with admission values for fetuin-A [area under the curve (AUC) 0.77 (0.68–0.86) at day 3 vs AUC 0.66 (0.54–0.79) at admission] and similarly for NT-proBNP [AUC 0.82 (0.73–0.92) at day 3 vs AUC 0.85 (0.76–0.93) at admission]. For the following analysis, we considered only day 3 values. The optimal cutoff values derived from ROC curves (Fig. 1) was 145 mg/L fetuin-A, close to the abnormal cutoff value, 300 ng/L for NT-proBNP, and 6 for the CADILLAC risk score. Sensitivity, specificity, negative predictive value, and positive predictive value to identify death were 80%, 67%, 97%, and 19%, respectively, with fetuin-A <140 mg/L. In patients with a CADILLAC risk score $<6$, low fetuin-A (OR 12, $P = 0.003$; Fig. 2A), and NT-proBNP $>300$ ng/L (OR 12, $P = 0.004$; Fig. 2B) were both predictive of death. In high-risk populations (CADILLAC score $\geq 6$), only low fetuin-A was associated with poor outcome (OR 7.4, $P = 0.001$; Fig. 2A). The death rate was 47% with low fetuin-A, and survival was 90% with fetuin-A $\geq 140$mg/L.

### Table 1. Population characteristics associated with low fetuin-A.\(^a\)

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<th>Fetuin-A at admission</th>
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<th>Fetuin-A at day 3</th>
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<tr>
<td></td>
<td>$\geq 140$ mg/L</td>
<td>$&lt; 140$ mg/L</td>
<td>$\geq 140$ mg/L</td>
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<tr>
<td>n</td>
<td>222</td>
<td>62</td>
<td>177</td>
</tr>
<tr>
<td>Age, years</td>
<td>60 (14)</td>
<td>66 (14)</td>
<td>58 (13)</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>86 (31)</td>
<td>74 (28)</td>
<td>89 (31)</td>
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<td>Admission heart failure, n (%)</td>
<td>35 (16)</td>
<td>18 (29)</td>
<td>26 (15)</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>51 (11)</td>
<td>49 (11)</td>
<td>52 (11)</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>5 (3–10)</td>
<td>5 (4–33)</td>
<td>11 (4–29)</td>
</tr>
<tr>
<td>NT-proBNP, ng/L</td>
<td>202 (50–548)</td>
<td>344 (139–1919)</td>
<td>210 (60–559)</td>
</tr>
<tr>
<td>CADILLAC risk score</td>
<td>2 (0–5)</td>
<td>3 (0–7)</td>
<td>2 (0–5)</td>
</tr>
<tr>
<td>Death</td>
<td>13 (5.8)</td>
<td>12 (19.4)</td>
<td>5 (2.8)</td>
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\(^a\) Data are mean (SD) or mean (range), unless noted otherwise.
Inflammation is a common feature of all different stages of atherosclerosis. Much evidence exists for the detrimental impact of proinflammatory cytokines (24, 25). Recent data consistently suggest that antiinflammatory markers may have similar importance and may be even more important than proinflammatory mediators (7, 8). Multi-variable analysis adjusted to CRP, NT-proBNP, and CADILLAC risk score demonstrates that risk for death after STEMI is dramatically increased when the antiinflammatory fetuin-A concentration at day 3 is low (<140 mg/L). Conversely, when the fetuin-A concentration is ≥140 mg/L, survival is excellent (97% overall), even in a high-risk population (90% when CADILLAC score ≥6).

Accumulating evidence shows that low fetuin-A concentrations are related to increased cardiovascular mortality in ESRD populations (19, 20). A high rate of mortality in patients undergoing dialysis is attributed to rapid atherosclerotic disease evolution resulting from impairment of calcium salt precipitation control and inflammatory imbalance, both related to decreased fetuin-A activities. In addition, a low fetuin-A concentration has a direct deleterious effect on myocardial function. Merx et al. (14) recently reported promotion of cardiac fibrosis, calcification, notably impaired diastolic function, and tolerance to ischemia as well as catecholamine resistance in the hearts of fetuin-A knockout mice. Our study reports the important role of fetuin-A in STEMI. The findings are strengthened by previous studies consistently demonstrating the determinant role of antiinflammatory mediators in acute coronary syndrome (ACS). Using a factorial analysis approach, Tziakas et al. (8) demonstrated that antiinflammatory clusters, including HDL cholesterol and IL-10, were a better predictor of recurrent ACS than inflammatory markers. Similarly, Heeschen et al. (7) reported that CRP concentration lost its significance when IL-10 was introduced into the multivariate analysis to predict death and nonfatal acute myocardial infarction after ACS. Inflammation is the determinant mechanism of plaque rupture in STEMI. After necrosis, inflammation will follow in the widespread myocardium (26), and its consequences can be favorable, leading to healing and restoration of function, or unfavorable, leading to acute cardiac rupture or chronic dilation, then heart failure. The role of antiinflammatory mediators in counteracting and modu-

<table>
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<th>Table 2. Independent correlates of 6-month death.</th>
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<td>Model 1</td>
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<td><strong>Fetuin-A as a continuous variable</strong></td>
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<tr>
<td>CADILLAC risk score</td>
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<td>Log peak cTnI</td>
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<td>Admission</td>
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<td>Log NT-proBNP</td>
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<td>Log fetuin-A</td>
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<tr>
<td>Day 3</td>
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<tr>
<td>Log NT-proBNP</td>
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<td>Log fetuin-A</td>
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<td><strong>Fetuin-A as a categorical variable</strong></td>
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<td>CADILLAC risk score</td>
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<td>Admission</td>
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<td>Log NT pro-BNP</td>
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<tr>
<td>Fetuin-A &lt;140 mg/L</td>
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<td>Day 3</td>
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<td>Log NT pro-BNP</td>
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<td>Fetuin-A &lt;140 mg/L</td>
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Fig. 1. ROC curves of significant predictors of death.
lating the inflammatory process appears crucial to avoid inadequate healing. As a consequence, imbalance between anti- and proinflammatory mediators could be deleterious (5, 6). Antiinflammatory mediators are commonly involved in the downregulation of proinflammatory cytokine production through separate mechanisms (3, 27).

Fetuin-A acts as a ubiquitous cofactor, usually required to opsonize cationic macrophage deactivating molecules (10). Physiologically, during inflammation, proinflammatory cytokines, whose production is usually inhibited by fetuin-A, will decrease fetuin-A liver synthesis (28). Decreased circulating fetuin-A concentrations will affect and limit activities of numerous antiinflammatory mediators, enabling amplification. In the setting of STEMI, a low fetuin-A concentration will facilitate the ongoing inflammatory process (11) and also the overproduction of cardiotoxic cytokines such as tumor necrosis factor (28, 29), which will expose patients to a high risk of LV remodeling and recurrence of ACS. The marked decrease of fetuin-A at day 3 (i.e., its synthesis inhibition) coincides with the peak of postinfarction inflammation (30), explaining the correlation of low fetuin-A concentrations, improved prognosis, and markers of the severity of necrosis at day 3 (Table 1). We believe that the fetuin-A concentration indicates the state of inflammatory imbalance, because it is the result of pro- and antiinflammatory cytokine interaction, and this crucial information, not provided by CRP and NT-proBNP, should be added to clinical risk score. As illustrated in Fig. 2, identification of high-risk patients who may require more intensive monitoring is significantly improved when low fetuin-A concentration is combined with CADILLAC risk score.

For several reasons the use of fetuin-A values to assess prognosis should be restricted to STEMI patients treated by PCI. First, in a recent cross-sectional study, Ix et al. (31) reported increased fetuin-A concentrations in patients with metabolic syndrome and atherogenic lipid profile. This paradoxical finding could be interpreted either as a consequence of increased fetuin-A liver synthesis induced by metabolic syndrome or as a U-shaped relationship between fetuin-A and cardiovascular risk. Second, the present study included only a limited number of patients with facilitated PCI. Because of the small number of these patients, the prognostic value of low fetuin-A should be interpreted with caution in this population. In addition, the study included only patients surviving at least 3 days after STEMI; therefore the prognostic value of fetuin-A for early mortality must still be defined. Third, because of the low number of patients and endpoints, our results may be subject to type II error for proinflammatory markers and type I for fetuin-A. To decrease the risk for type I error, however, we used a stepwise multivariate regression method, and the results were consistent with previous reports. Finally, normal fetuin-A concentration was defined in a small control population and should be verified in a larger cohort.

In conclusion, a low concentration of the antiinflammatory mediator fetuin-A (<140 mg/L) is strongly associated with death within 6 months in patients with STEMI. Thus fetuin-A measurement used in addition to NT-proBNP measurement and CADILLAC risk score enables more accurate identification of high-risk patients.

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