# Association between beta2-glycoprotein I plasma levels and the risk of myocardial infarction in older men

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**von Willebrand factor (VWF) serves as adhesive surface for platelets to adhere to the vessel wall. We have recently found that beta2-glycoprotein I is able to inhibit platelet binding to VWF, indicating a role in the pathophysiology of arterial thrombosis. In the present study, we investigated whether differences in beta2-glycoprotein I plasma levels influence the risk of myocardial infarction. We have measured beta2-glycoprotein I and VWF antigen levels in 539 men with a first myocardial infarction and in 611 control subjects. Although we did not find a profound effect of beta2-glycoprotein I plasma levels on myocardial infarction in the overall population, we found a dose-dependent protective effect of increasing beta2-glycoprotein I plasma levels on myocardial infarction in men 60 years and older. In this age group, we found an odds ratio of 0.41 (95% confidence interval, 0.22-0.74) for high beta2-glycoprotein I levels compared with low levels. High plasma levels of beta2-glycoprotein I remained protective for myocardial infarction despite high levels of VWF. To conclude, high circulating levels of beta2-glycoprotein I appeared to be associated with a reduced risk of myocardial infarction in elderly men. In vivo experiments are needed to investigate the exact contribution of beta2-glycoprotein I on the pathophysiology of myocardial infarction. (Blood. 2009;114:3656-3661)**



## Introduction

Myocardial infarction is a thrombotic disorder with a high incidence in Western society. It is usually caused by the formation of occlusive thrombi on atherosclerotic lesions. Adhesion of platelets to ruptured atherosclerotic lesions is a multistep process involving von Willebrand factor (VWF) present in the plaque and the glycoprotein Ib (GPIb)/IX/V complex on platelets.[1] Under high shear stress, the VWF/GPIb/IX/V interaction is crucial for the adhesion of platelets to VWF bound to subendothelial collagen.[2] VWF is a multimeric protein that is produced by both platelets and endothelial cells. In platelets, VWF is stored in the alpha-granules and in endothelial cells VWF is stored in so-called Weibel-Palade bodies.[3] Its plasma antigen level is approximately 40 nM and is influenced by both inherited factors (ABO blood groups, mutations in the VWF gene) and acquired factors, such as diabetes.[4] Although it is still subject of debate, it is assumed that high plasma levels of VWF are associated with an increased risk of arterial thrombosis.[5,6]

Platelet binding to VWF is mediated by the A1 domain of VWF. VWF present in the circulation and GPIb of platelets do not interact with each other; however, immediately after binding to the vessel wall, VWF supports the adhesion of platelets. A conformational change in VWF results in the exposure of neoepitopes that is required before GPIb is able to interact with VWF.[7] The conformational change can be induced by nonphysiologic agents, such as ristocetin, or can be the result of mutations within VWF.[8,9] We recently found that beta2-glycoprotein I (beta2GPI), a plasma protein known for its role in the antiphospholipid syndrome, influences platelet-VWF interaction in vitro by binding to the A1 domain of VWF when it has obtained its GPIb-binding conformation.[10] As a result, the adhesion of platelets to VWF is hampered, implying a role for beta2GPI in platelet adhesion and thus in the development of arterial thrombosis.

In this study, we investigated whether variations in plasma levels of beta2-glycoprotein I influence the risk of myocardial infarction. We measured beta2-glycoprotein I plasma levels in a large case-control study (Study of Myocardial Infarction Leiden [SMILE]) comprising 539 men with a first myocardial infarction and 611 control men. In addition, we investigated the interrelationship of levels of VWF and beta2-glycoprotein I and the risk of myocardial infarction.

## Methods

### Patients and control subjects

Plasma samples were used from a case-control study, SMILE, of which details were published previously.[11] In short, the control subjects consisted of 646 men who had a minor orthopedic intervention between January 1990 and January 1996. As a consequence, these men, who had undergone minor

Submitted March 25, 2009; accepted August 3, 2009. Prepublished online as *Blood* First Edition paper, August 25, 2009; DOI 10.1182/blood-2009-03-212910.

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**Table 1. Characteristics of patients with myocardial infarction and control subjects**

| | Patients, N = 539 | Control subjects, N = 611 |
|---|---|---|
| Mean age, y (SD) | 56.4 (9.0) | 57.5 (10.6) |
| **Smoking, n (%)** | | |
| No | 206 (38.2) | 410 (67.1) |
| Yes | 333 (61.8) | 201 (32.9) |
| **Alcohol, n (%)** | | |
| Never | 83 (15.4) | 61 (10.0) |
| Occasionally | 24 (4.5) | 21 (3.4) |
| Regularly | 432 (80.1) | 529 (86.6) |
| **Quetelet index, kg/m$^2$, n (%)*** | | |
| Less than 30 | 448 (83.3) | 513 (84.1) |
| 30 or more | 90 (17.6) | 97 (15.9) |
| **Diabetes, n (%)** | | |
| Absent | 513 (95.2) | 590 (96.6) |
| Present | 26 (4.8) | 21 (3.4) |
| **Hypertension, n (%)** | | |
| Absent | 434 (80.5) | 506 (82.8) |
| Present | 105 (19.5) | 105 (17.2) |
| **Hypercholesterolemia, n (%)** | | |
| Absent | 527 (97.8) | 600 (98.2) |
| Present | 12 (2.2) | 11 (1.8) |

Data refer to the period prior to myocardial infarction, apart from the Quetelet index.

*For one patient and one control subject, the Quetelet index was missing.

orthopedic intervention, were treated with anticoagulants in an anticoagulation clinic. These control men were recruited for the SMILE study after discontinuation of anticoagulant treatment. The characteristics of patients and control subjects are indicated in Table 1 and, as expected, differed between the 2 groups.

In this study, 560 men were included, aged between 18 and 70 years, and consecutively diagnosed with a first episode of myocardial infarction between January 1990 and January 1996. The control group consisted of 646 men who had a minor orthopedic intervention between January 1990 and May 1996. Both patient and control subjects were living in the same area and were born in The Netherlands, although no data on ethnicity are known. The control subjects did not have a history of myocardial infarction. Control subjects were frequency-matched to patients on 10-year age groups for efficiency reasons. All participants completed a questionnaire and an interview took place before blood draw. Questions referred to (former) smoking habits and alcohol use and diabetes. In addition, data on patients' diabetes and medication use before myocardial infarction were retrieved from discharge letters. A person was classified as having hypertension or hypercholesterolemia when he was prescribed specific medications for these conditions. The Quetelet index was derived by dividing weight (kilograms) by squared height (meters). The latter was measured after the interview in which questions referred to current medication use, including aspirin.

Fasting venous blood was drawn into tubes containing 0.109 M trisodium citrate as anticoagulant. Blood in the first tube was allowed to clot and the serum was used for measuring total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels. Total cholesterol and triglyceride levels were measured using enzymatic assays adapted to a Hitachi 747 (Boehringer Mannheim), and HDL cholesterol level was measured on a Hitachi 911 (Boehringer Mannheim). Blood taken in 0.109 M trisodium citrate was centrifuged for 10 minutes at 3000*g* at room temperature. The citrated plasma was aliquoted in multiple tubes and immediately stored at −80°C. The median time between myocardial infarction and blood collection was 2.6 years, and between orthopedic intervention and blood collection was 2.9 years, both with a minimum of 6 months. Approval for these studies was obtained from the Medical Ethics Committee of the Leiden University Medical Center. All participants provided written informed consent in accordance with the Declaration of Helsinki.

### ELISAs for VWF and beta2GPI

Beta2GPI antigen levels were detected by a sandwich-type enzyme-linked immunosorbent assay (ELISA) as previously described.[12] For detection, we used monoclonal antibody 2B2 with affinity for beta2GPI regardless of conformation that detects intact beta2GPI as well as nicked beta2GPI (kind gift of Prof A. Tincani, Brescia Hospital and University). Monoclonal antibody 2B2 was diluted in a Tris-buffered saline (TBS), pH 7.4, to reach a final concentration of 3 μg/mL and coated overnight at 4°C to an ELISA plate (Costar). After washing 3 times with TBS/0.1% Tween, the ELISA plates were incubated with a 3% bovine serum albumin (BSA)/TBS solution for 1 hour at 37°C. The plates were washed 3 times and subsequently incubated with plasma diluted 1:1000 in 3% BSA/TBS/0.1% Tween for 1 hour at 37°C. Serial dilutions of normal pool plasma (calibrated against purified plasma-derived beta2GPI) derived from 50 healthy volunteers were used as reference standard. Then, the plates were washed and incubated with a polyclonal rabbit anti–human beta2GPI antibody (Kordia) with a final concentration of 5 μg/mL diluted in 3% BSA/TBS/0.1% Tween. This was followed by incubation with a peroxidase-labeled goat anti–rabbit antibody (DAKO). Staining was performed using an ortho-phenylenediamine solution (4 mg/mL ortho-phenylenediamine diluted in 0.1 M $NaH_2PO_4.2H_2O$/0.1 M $Na_2HPO_4.H_2O$). The coloring reaction was stopped with the addition of 1 M $H_2SO_4$, and absorbance was measured at 490 nm. Results are displayed as micrograms per milliliter.

The VWF ELISA was performed according to the manufacturer's instructions (Diagnostica Stago).[13] Both VWF and beta2GPI levels were measured in 539 of 560 patients and 611 of 646 control subjects, from whom plasma samples were available.

### Statistical analysis

Differences in continuous variables between 2 groups were shown as a mean difference with a 95% confidence interval (CI). An interval of a mean difference that includes null indicates no difference at a significance level of 5% or less. Mann-Whitney was used to test the difference between median values. As estimates of relative risk, we calculated the odds ratio (OR) for quartiles of protein levels with 95% CIs. When this interval did not include unity, the OR was different from unity at a significance level of 5% or less. Quartiles were based on the distribution of control subjects. The lowest quartile was used as a reference category for the OR. Unconditional logistic regression was performed to adjust for age and to further adjust for VWF, total cholesterol, HDL cholesterol, log-transformed triglyceride levels, Quetelet index, diabetes (categoric), and hypertension (categoric). For all calculations, SPSS for Windows Version 14.0 (SPSS) statistical package was used.

## Results

Beta2GPI antigen levels were determined in 539 myocardial infarction patients and 611 control subjects. Characteristics are shown in Table 1. Mean age of patients was 56.4 years (5th-95th percentiles: 40.4-68.8) and of control subjects was 57.5 years (5th-95th percentiles: 35.3-72.1). In Table 2, the association of cardiovascular risk factors with beta2GPI antigen levels in control subjects is shown. No associations were found between smoking, alcohol use, and beta2GPI antigen levels (Table 2). The presence of obesity (≥ 30 kg/m$^2$), diabetes, and hypertension and high levels of cholesterol may be associated with higher beta2GPI antigen levels, but as CIs were wide and include null values, the differences may be due to chance. High HDL cholesterol levels may be associated with low beta2GPI antigen levels. In contrast, increased levels of triglycerides were associated with high levels of beta2GPI. Of the 611 control subjects, 35 were using aspirin before the blood draw. There were no differences in beta2GPI levels between aspirin users and nonusers (mean difference, −0.3 μg/mL; 95% CI, −28 to 27 μg/mL).

**Table 2. Cardiovascular risk factors among 611 control subjects and the association with beta2GPI**

| | Number | Beta2GPI, Mean CI | Difference between means (95% CI) |
|---|---|---|---|
| **Smoking** | | | |
| No | 410 | 217.8 | |
| Yes | 201 | 221.1 | 3.3 (−10.3 ↔ 16.9) |
| **Alcohol** | | | |
| Never | 61 | 221.0 | |
| Occassionally | 21 | 212.6 | −8.4 (−49.6 ↔ 32.9) |
| Regularly | 529 | 220.2 | −0.8 (−22.0 ↔ 20.4) |
| **Quetelet index, kg/m²*** | | | |
| Less than 30 | 513 | 218.0 | |
| 30 or more | 97 | 229.5 | 11.5 (−5.9 ↔ 28.9) |
| **Diabetes** | | | |
| Absent | 590 | 219.6 | |
| Present | 21 | 230.9 | 11.3 (−23.8 ↔ 46.3) |
| **Hypertension** | | | |
| Absent | 506 | 217.9 | |
| Present | 105 | 230.3 | 12.4 (−4.5 ↔ 29.3) |
| **Total cholesterol, mmol/L** | | | |
| Less than 5.02 | 120 | 209.2 | |
| 5.02-5.57 | 122 | 210.0 | 0.8 (−19.1 ↔ 20.7) |
| 5.58-6.08 | 124 | 227.9 | 18.7 (−0.3 ↔ 37.8) |
| 6.09-6.79 | 120 | 216.5 | 7.3 (−11.5 ↔ 26.1) |
| 6.80 or more | 125 | 235.7 | 26.5 (−5.9 ↔ 47.2) |
| **HDL cholesterol, mmol/L*** | | | |
| Less than 1.06 | 118 | 229.3 | |
| 1.06-1.22 | 119 | 219.4 | −9.9 (−30.2 ↔ 10.5) |
| 1.23-1.38 | 125 | 212.9 | −16.4 (−36.1 ↔ 3.3) |
| 1.39-1.61 | 125 | 223.9 | −5.4 (−27.4 ↔ 16.8) |
| 1.62 or more | 123 | 215.4 | −13.9 (−34.5 ↔ 6.8) |
| **Triglycerides, mmol/L** | | | |
| Less than 0.83 | 122 | 205.4 | |
| 0.83-1.09 | 126 | 209.3 | 3.9 (−15.7 ↔ 23.5) |
| 1.10-1.41 | 121 | 226.4 | 21.0 (1.0 ↔ 40.9) |
| 1.42-2.03 | 124 | 224.1 | 18.7 (−1.3 ↔ 38.7) |
| 2.04 or more | 118 | 235.7 | 30.3 (9.1 ↔ 51.4) |

* For one control subject, the Quetelet index and HDL cholesterol were missing.

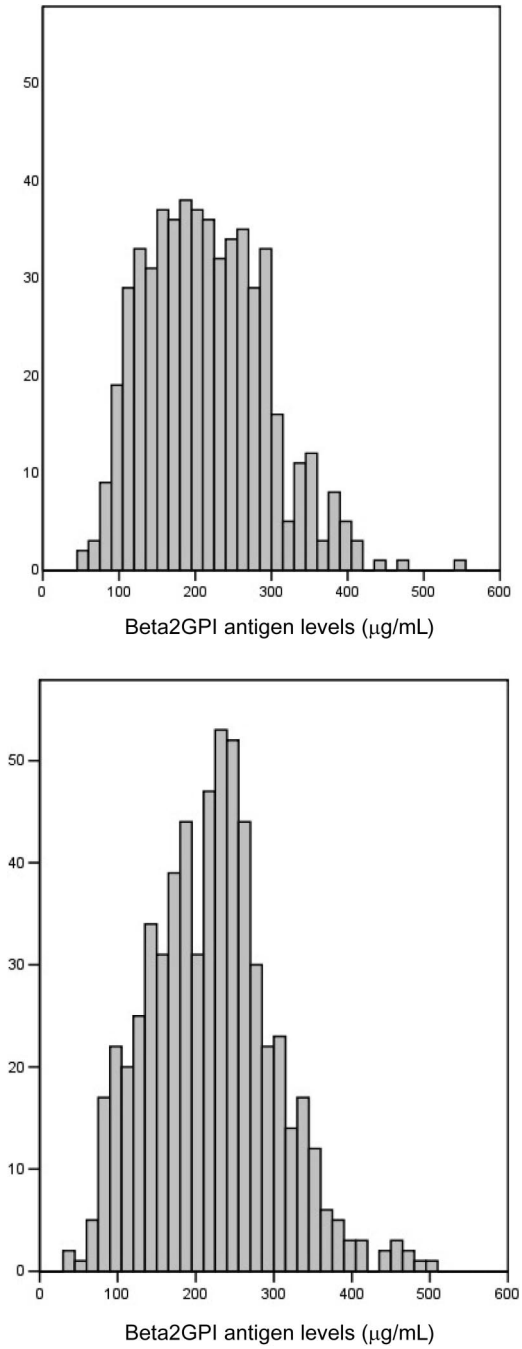
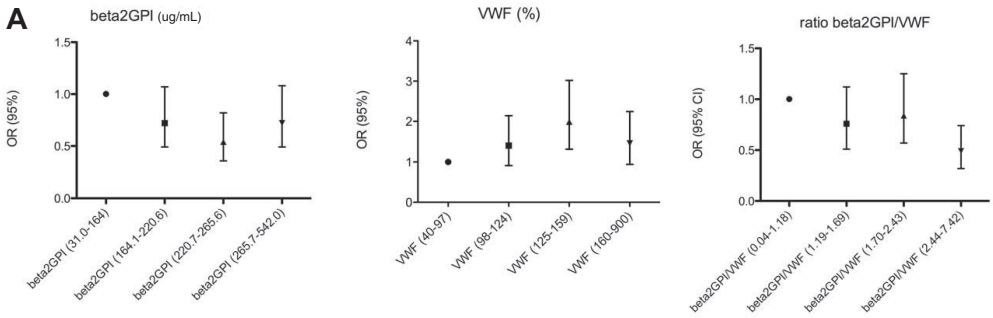



**Figure 1. Distribution of beta2GPI levels in 539 men with a first myocardial infarction and 611 control subjects.** (Top panel) The distribution of beta2-glycoprotein plasma levels of 539 men with a first myocardial infarction (mean plasma level of 214 μg/mL [5th-95th percentile, 100-354 μg/mL]). (Bottom panel) The distribution of beta2-glycoprotein plasma levels of 611 control subjects (mean plasma level of 220 μg/mL [5th-95th percentile, 94-354 μg/mL]).

Beta2GPI antigen levels were widely distributed among men with a myocardial infarction and in control subjects (Figure 1). There was no overall difference between mean beta2GPI plasma level of the myocardial infarction patients (214 μg/mL) and control subjects (220 μg/mL; mean difference, −6 μg/mL; 95% CI, −15 to 3 μg/mL). Using the lowest quartile of beta2GPI plasma level as a reference group, we calculated the OR for myocardial infarction of each quartile. Although for most quartiles risks of myocardial infarction appeared to be reduced compared with the reference category, we did not observe a clear association of beta2GPI levels with myocardial infarction, that is, no graded effect over quartiles (Figure 2A). Adjustment for VWF levels did not change the ORs.

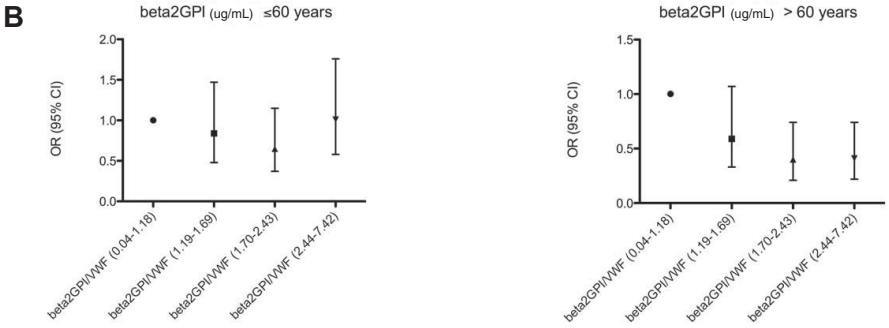
One hundred twenty-one patients and 35 control subjects were using aspirin before the blood draw. Excluding these people resulted in similar ORs for beta2GPI as in the overall group; ORs after age adjustment were 0.88 (95% CI, 0.62-1.24) for the second quartile, 0.60 (95% CI, 0.42-0.87) for the third quartile, and 0.84 (95% CI, 0.60-1.20) for the highest quartile compared with the lowest quartile. Adjustment for cardiovascular risk factors further reduced these ORs.

High VWF levels were associated with an increased risk of myocardial infarction (Figure 2A), as shown before.[13] As beta2GPI inhibits binding of platelets to VWF, we hypothesized that high levels of VWF could negatively influence the results obtained for beta2GPI plasma levels and myocardial infarction. Therefore we investigated the influence of VWF on the effect of beta2GPI by comparing the beta2GPI/VWF ratio between both groups. We found a difference between median beta2GPI/VWF ratio for myocardial infarction patients and control subjects (1.57 vs 1.69; Mann-Whitney, $P < .01$). In addition, the risk of myocardial infarction was calculated for each quartile using the lowest beta2GPI/VWF quartile as reference group. An increase in




| | Patients N=539 | Control N=611 | OR (95% CI)** | OR (95% CI) *** |
|---|---|---|---|---|
| **BetaGPI (µg/mL)** | N (%) | N (%) | | |
| 31.0 – 164.0 | 161 (29.9) | 153 (25.0) | 1* | 1* |
| 164.1 – 220.6 | 135 (25.0) | 153 (25.0) | 0.86 (0.62 – 1.18) | 0.72 (0.49- 1.07) |
| 220.7 – 265.6 | 104 (19.3) | 153 (25.0) | 0.66 (0.47 – 0.92) | 0.54 (0.36 – 0.82) |
| 265.7 – 542.0 | 139 (25.8) | 152 (25.0) | 0.89 (0.64 – 1.22) | 0.72 (0.49 – 1.08) |
| | | | | |
| **VWF (%)** | | | | |
| 40-97 | 104 (19.3) | 153 (25.0) | 1* | 1* |
| 98-124 | 126 (23.4) | 152 (24.9) | 1.30 (0.92 – 1.84) | 1.40 (0.91 – 2.14) |
| 125-159 | 164 (30.4) | 154 (25.2) | 1.73 (1.23 – 2.43) | 1.99 (1.31 – 3.02) |
| 160-900 | 145 (26.9) | 152 (24.9) | 1.60 (1.13 – 2.28) | 1.45 (0.94 – 2.24) |
| | | | | |
| **Beta2GPI/VWF ratio** | | | | |
| 0.04 – 1.18 | 169 (31.4) | 153 (25.0) | 1* | 1* |
| 1.19 – 1.69 | 138 (25.6) | 153 (25.0) | 0.82 (0.59 – 1.12) | 0.76 (0.51 – 1.12) |
| 1.70 – 2.43 | 129 (23.9) | 152 (25.0) | 0.75 (0.44 – 1.04) | 0.84 (0.57 – 1.25) |
| 2.44 – 7.42 | 103 (19.1) | 153 (25.0) | 0.58 (0.42 – 0.82) | 0.49 (0.32 – 0.74) |

* reference group
** adjusted for age
*** adjusted for age, total cholesterol, HDL cholesterol, lg(triglycerides), quetelet index, diabetes, hypertension as measured at time of blood draw




| | Patients | Control | OR (95% CI)** | OR (95% CI) *** |
|---|---|---|---|---|
| **BetaGPI (µg/mL)** | N (%) | N (%) | | |
| | | | | |
| **≤60 years** | | | | |
| 31.0 – 164.0 | 88 (27.9) | 95 (29.5) | 1* | 1* |
| 164.1 – 220.6 | 73 (23.3) | 78 (24.2) | 0.99 (0.64 – 1.53) | 0.84 (0.48 – 1.47) |
| 220.7 – 265.6 | 63 (20.0) | 82 (25.2) | 0.81 (0.52 – 1.25) | 0.65 (0.37 – 1.15) |
| 265.7 – 542.0 | 91 (28.9) | 67 (20.8) | 1.42 (0.93 – 2.19) | 1.01 (0.58 – 1.76) |
| | | | | |
| **>60 years** | | | | |
| 31.0 – 164.0 | 73 (32.6) | 58 (20.1) | 1* | 1* |
| 164.1 – 220.6 | 62 (27.7) | 75 (26.0) | 0.74 (0.45 – 1.22) | 0.59 (0.48 – 1.07) |
| 220.7 – 265.6 | 41 (18.3) | 71 (24.6) | 0.49 (0.29 – 0.83) | 0.40 (0.21 – 0.74) |
| 265.7 – 542.0 | 48 (21.4) | 85 (29.4) | 0.47 (0.28 – 0.78) | 0.41 (0.22 – 0.74) |
| | | | | |

* reference group
** adjusted for age
*** adjusted for age, total cholesterol, HDL cholesterol, lg(triglycerides), quetelet index, diabetes, hypertension as measured at time of blood draw

**Figure 2. Risk of myocardial infarction and beta2GPI plasma levels.** (A) Risk of myocardial infarction with increasing quartile of beta2GPI antigen, VWF levels, and the ratio of beta2-GPI/VWF. (B) Risk of myocardial infarction with increasing quartile of beta2GPI antigen, for men younger than and older than 60 years. Error bars represent 95% confidence interval.

**Table 3. Risk of myocardial infarction in the total group with different levels of beta2GPI and VWF**

| Beta2GPI | VWF | Patients | Control subjects | OR (95% CI) |
|---|---|---|---|---|
| **Overall** | | N = 539 | N = 611 | |
| Low | Low | 446 | 499 | 1* |
| Low | High | 46 | 53 | 1.00 (0.68-1.59) |
| High | Low | 42 | 52 | 0.92 (0.60-1.41) |
| High | High | 5 | 7 | 0.81 (0.26-2.59) |
| **More than 60 y** | | n = 224 | n = 289 | |
| Low | Low | 181 | 217 | 1* |
| Low | High | 28 | 39 | 0.95 (0.56-1.64) |
| High | Low | 14 | 29 | 0.59 (0.30-1.18) |
| High | High | 1 | 4 | 0.30 (0.03-2.74) |

Low indicates below 90th percentile (based on control subjects: beta2GPI 326 μg/mL, VWF 199%); high, above 90th percentile; and OR, odds ratio adjusted for age.

*Reference group.

beta2GPI/VWF ratio was associated with a lower risk of myocardial infarction, especially for the highest quartile (OR, 0.49 [95% CI, 0.32-0.74]; Figure 2A).

As young men have a different risk profile for myocardial infarction than older men, we decided to divide the participants into different 10-year age groups and calculated the OR for beta2GPI levels. In the age groups younger than 40 years, 40 to 49 years, and 50 to 59 years, high levels of VWF were still associated with myocardial infarction (data not shown), but no clear associations were found for levels of beta2GPI and myocardial infarction. Overall, in men younger than 60 years, no associations were found (Figure 2B). In men older than 60 years, we found an inverse association for beta2GPI as the ORs for the highest quartiles was 0.47 (95% CI, 0.28-0.78) and for the third quartile was 0.49 (95% CI, 0.29-0.83) compared with the lowest quartile (Figure 2B). Adjustment for cardiovascular risk factors further reduced these ORs to 0.41 (95% CI, 0.22-0.74) for the highest and 0.40 (95% CI, 0.21-0.74) for the third quartile. Excluding aspirin users (47 patients, 18 control subjects) resulted in similar ORs as including these aspirin users.

The association between beta2GPI levels, VWF levels, and myocardial infarction was studied in more detail by investigating combinations of high and low levels of both proteins and their association with myocardial infarction. For both beta2-glycoprotein I and VWF, protein levels below the 90th percentile were defined as low levels and protein levels above the 90th percentile, as high levels. We did not find an association between any of the combinations of VWF and beta2GPI levels and myocardial infarction (Table 3). Although numbers are small and conclusions should be made with caution, in men older than 60 years, high levels of beta2-glycoprotein I appeared to be protective against myocardial infarction in people with either low levels (OR, 0.59; 95% CI, 0.30-1.18) or high levels (OR, 0.30; 95% CI, 0.03-2.74) of VWF compared with people with low levels of both beta2-glycoprotein I and VWF (Table 3).

As age seems to have an influence on the protective effect of beta2GPI on myocardial infarction, we studied the relationship between age and beta2GPI plasma levels, and between age and the beta2GPI/VWF ratio among control subjects. The beta2GPI plasma level increased every 10 years with 12.3 μg/mL (95% CI, 6.4-18.2). Despite this gradual increase in the beta2GPI plasma level, the beta2GPI/VWF ratio decreased −0.06 every 10 years (95% CI, −0.13 to 0.02), suggesting that the VWF plasma level increases more with age than beta2GPI.

## Discussion

Several reports have suggested an anticoagulant role for beta2GPI in hemostasis.[14,15] We have recently postulated a role for beta2GPI in VWF-dependent hemostasis as in vitro beta2GPI inhibited the VWF-platelet interaction.[10] In the present study, we observed an inverse relationship between beta2GPI plasma levels and myocardial infarction in older men. Taking VWF into account, we found that an increase in the beta2GPI/VWF ratio was accompanied with a reduced risk of myocardial infarction, especially in men older than 60 years. Together with the previous study in which we have shown that beta2GPI and VWF interact with each other, our data might suggest that beta2GPI may be a factor involved in the pathophysiology of myocardial infarction.[10]

Although high levels of VWF have been shown to be associated with an increased risk of arterial thrombosis, there is no consensus on this issue. Differences in beta2GPI levels might explain the contrasting study results of VWF level on the risk on myocardial infarction. Furthermore, differences in age distributions of the populations under study may contribute to the different results. Based on our data, beta2GPI levels appeared to influence the risk of high levels of VWF of myocardial infarction, especially in men older than 60 years. More importantly, high levels of beta2GPI seem to decrease the risk of myocardial infarction more than VWF increased the risk. It must be said that this analysis was done in a small subpopulation and therefore any conclusion must made with caution. Although the present study includes a large number of participants, and more than 200 patients and 300 control subjects are older than 60 years, these results need to be confirmed by other studies. Furthermore, as can be seen in Figure 1 there is a wide distribution of the beta2GPI plasma levels. This is in line with previous reports and might account for differences in mean beta2GPI plasma levels between different populations. In addition, no association was found between beta2GPI plasma levels and recurrent major arterial cardiovascular events among men who had a first myocardial infarction (data not shown).

Beta2GPI plasma levels increased with age, a finding that is in line with a previous report.[16] As elderly people suffer more regularly from pathologic conditions, the rise in beta2GPI levels might be a result of increased production or decreased clearance. In this respect, it is interesting to note that VWF also increases with age.[17] In our study, VWF showed a stronger increase in plasma levels than beta2GPI over a lifetime, which is reflected by lower beta2GPI/VWF ratio at higher age. As the elderly are more prone to myocardial infarction due to increased atherosclerosis, the reduced beta2GPI/VWF ratio might be causative for the increased risk of myocardial infarction. In this respect, it has to be noted that an increase in VWF can be secondary to the occurrence of a myocardial infarction. Although blood samples were drawn at least 6 months after the event (median, 2.6 years), we cannot completely rule out this possibility. Furthermore, our study includes only patients who survived, and those who died may have had very high levels. As external factors such as patient delay and delay in providing effective assistance influence mostly survival in patients with a myocardial infarction, we do not think that survival bias influenced our results, but we cannot completely rule out this possibility.

Beta2GPI is one of the major cofactors in the antiphospholipid syndrome. Recently, we have shown that anti-beta2GPI antibodies were able to abolish the inhibitory actions of beta2GPI on VWF-dependent platelet adhesion. In the present study, we have

also measured anti-beta2GPI antibodies in both the patient population and the control population. Sixteen percent of the patients and 12% of the control subjects were positive for anti-beta2GPI antibodies. We have found that the presence of anti-beta2GPI antibodies did not influence our results (data not shown).

In conclusion, in line with our previous report on the inhibitory properties of beta2GPI on VWF-dependent platelet adhesion, our findings indicate that beta2GPI appears to have a protective effect on myocardial infarction in men older than 60 years.[10] In the future, besides other studies confirming our results, in vivo experiments are needed to explain both the physiologic and pathophysiologic role of beta2GPI in hemostasis.

## Acknowledgments

We thank the cardiologists of the Departments of Cardiology, Leiden University Medical Center and the Diaconessenhuis Leiden and F. J. M. van der Meer, head of the Leiden Anticoagulant Clinic, for their kind cooperation; T. Visser for drawing blood samples; J. J. Schreijer and I. de Jonge for their secretarial and administrative support; and J. J. J. Hulstein for technical assistance in measurement of beta2GPI levels. We also express our gratitude to all people who participated in the "Study of Myocardial Infarction Leiden."

This research was supported by the Netherlands Heart Foundation (grant no. 92.345 and grant no. 2006T053).

## Authorship

Contribution: B.d.L. performed research and wrote the paper; P.G.d.G. and F.R.R. designed the study, supervised the project, and wrote the paper; R.H.W.M.D. and K.M. wrote the paper; R.T.U. performed research; and C.J.M.D. designed the study, analyzed the data, and wrote the paper. The authors had full access to the data and take responsibility for its integrity. All authors have read and agreed to the paper as written.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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