Platelet Aggregation and HDL Cholesterol Are Predictive of Acute Coronary Events in Heart Transplant Recipients

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Background Sudden death (SD) and acute myocardial infarction (AMI) are the main complications limiting long-term survival after heart transplantation (HT). They are unpredictable and, at present, unpreventable. Platelet aggregation (PA) has recently emerged as a significant prognostic indicator in nontransplanted coronary disease patients. The main purpose of the present study was to evaluate to what extent PA could predict SD and AMI in long-term survivors of HT independently of serum lipid levels.

Methods and Results We studied 207 patients. All received triple immunosuppressive therapy. During follow-up, the incidence of SD and AMI was determined, and the independent role of PA as predictor was evaluated with other usual risk factors by a Cox multivariate regression model. There were 11 SDs and 14 AMIs after an average follow-up of 642 days, giving an average incidence rate of 7.3 events per year per hundred patients. By univariate analysis, the most potent predictors were ADP-induced platelet aggregation (positive association) and total cholesterol (negative association). Age and length of time since transplant were not predictors. By multivariate analysis, only the secondary wave of ADP-induced platelet aggregation (\(P=.001\)) and high-density lipoprotein cholesterol (\(P=.03\)) were independent predictors. The relative risk of SD or AMI based on a comparison between patients with high (>36%) or low (<36%) ADP-induced platelet aggregation was 4.3 (95% confidence interval, 1.9 to 9.5, \(P=.001\)).

Conclusions This study provides the first demonstration of an association between increased platelet aggregation and subsequent SD or AMI in HT recipients. It suggests that platelets and thrombosis also are implicated in the pathogenesis of AMI and SD in HT recipients. Identification of a safe and effective antiplatelet therapy should be actively pursued.

Circulation. 1994;89:2590-2594.

Key Words • transplantation • myocardial infarction • death, sudden • cholesterol • platelets

Accelerated coronary heart disease (CHD), the main barrier to long-term survival after heart transplantation, is characterized by the rapid (within a few months) development of a concentric intimal thickening and a high incidence of CHD acute complications such as sudden death (SD) and fatal and nonfatal acute myocardial infarction (AMI).1-3 Presently, these events cannot be either predicted or prevented. In particular, antiplatelet treatment, the effect of which on reducing the incidence of CHD and stroke is well established in nontransplanted patients,4 did not appear to be efficient in heart transplant (HT) recipients.5,6 A possible explanation for this difference is that the drug used, aspirin, does not significantly reduce platelet aggregation in HT recipients, contrary to what occurs in nontransplanted patients.7 This suggests that in HT recipients, the mechanism of the enhanced platelet aggregation we reported previously8 is different from that observed in usual coronary disease patients.9

We have recently shown that enhanced platelet aggregation and coronary thrombosis may explain the high prevalence of CHD after heart transplantation.10 The aim of the present study was to determine to what extent platelet aggregation could predict subsequent SD and AMI in HT recipients independently of serum lipids.

Methods

Patients and Follow-up

The cohort consisted of HT recipients <70 years of age, clinically stable and ambulatory, transplanted more than 6 months earlier, consecutively referred to the outpatient clinic of the Institut National de la Santé et de la Recherche Médicale (INSERM, Unit 63) in Lyon between October 1988 and February 1992. The protocol was approved by the local Ethical Committee, and informed consent was granted by all patients. Exclusion criteria were (in addition to transplant of less than 6 months) overt heart failure (New York Heart Association classes III and IV), recent acute rejection episode, or malignant disease. All the patients were treated by the standard triple-immunosuppressive treatment of cyclosporine, prednisone, and azathioprine. Dosages of cyclosporine were adapted so that trough cyclosporine blood levels were >100 ng/mL with respect to kidney function. None of them were given aspirin, nonsteroid anti-inflammatory drugs, other antiplatelet drugs, or hypolipidemic agents. At each visit, patients were examined by a cardiologist and ECGs were recorded. SD and fatal and nonfatal AMI were the two major acute CHD outcomes considered and combined in the statistical analysis. SD was defined as death occurring without previous symptoms or unwitnessed, unexpected, and otherwise unexplained. The diagnosis of AMI was made according to World Health Organization criteria, ECG Minnesota Code, and confirma-
Clinical and Laboratory Data Evaluation

Arterial pressure was measured by an automatic sphygmomanometer in a fasting state and before venipuncture. Blood samples were drawn without stasis from an antecubital vein through a 21-gauge butterfly needle, always between 8:30 and 10:30 AM, thus about 12 hours after the previous cyclosporine dose. For the platelet function study, the blood was anticoagulated with 3.8% trisodium citrate (9:1, vol/vol) and immediately centrifuged to obtain platelet-rich (PRP) and platelet-poor (PPP) plasma. Two 10-ml samples were taken without anticoagulant for biochemical and lipid determinations in serum. Creatinine, glucose, and uric acid were automatically determined by a Chem1 Bayer Diagnostic Analyzer; total cholesterol and triglycerides were measured by an enzymatic assay kit (Biomérieux); and high-density lipoprotein cholesterol (HDL-C) after a dextran sulfate precipitation procedure. Low-density lipoprotein cholesterol (LDL-C) was estimated with the Friedewald formula. Total serum apoprotein B100 and apoprotein A1 were determined by immunonephelometry (Behring). All measurements were standardized against reference materials supplied by Biotrol Laboratories.

Platelet aggregation was performed on a recording aggregometer (Rubel-Renaud) in PRP, with a platelet count adjusted to 300,000/ml by dilution with PPP as described in previous reports. For the determinations, 500 μl PRP was warmed at 37°C for 2 minutes with stirring at 1100 rpm. Then 100 μl of the aggregating agent diluted in complete Tyrode’s solution (pH 7.4) was added. These agents were thrombin (human horphylized; Sigma Chemical Co; final concentration in PRP, 0.04 National Institutes of Health U/ml) and ADP (from equine muscle; Sigma; final concentration, 0.2×10⁻³ mol/L).

The aggregometer was adjusted for each sample so that PRP gave no light transmission and PPP gave 100% light transmission. The extent of aggregation by each agent was evaluated as the percentage of maximum difference between PRP and PPP. The value retained was the mean of three measurements made with each agent. The delay between blood sampling and the platelet aggregation test was constant for each agent (between 70 and 90 minutes). The tracings obtained were analyzed by two independent observers.

Previous studies have evaluated the extent of intrapatient variability of aggregation with time in transplanted and nontransplanted patients. The reproducibility was highly satisfactory, one evaluation (performed in triplicate) of platelet aggregation for each subject of the cohort was used for its relation to future cardiac events.

Statistical Methods

In addition to platelet aggregation, the following factors were included in the analysis: age, length of time since transplant, lipid levels, and drug treatment. The Cox proportional-hazards regression model was used to estimate the effects of the factors recorded at baseline as predictors of subsequent acute CHD events. Individual associations were tested univariately by construction of separate regression models for each factor. Event-free intervals were calculated with the date of enrollment as the starting point and the date of SD or AMI as the end point. Each variable was then introduced into the Cox model as a quadratic covariate after it was established that their relations with the hazards of acute CHD events were continuous. A multivariable Cox regression model of time to event was used to assess the relative contribution of each factor as a predictor. Relative risks and their corresponding 95% confidence intervals are reported for each variable. The log-rank test was used to compare the time-to-event rate between patients with “high” or “low” platelet aggregation as described in a previous study. The 36% cutoff point we used to separate “high” and “low” platelet aggregation patients was identical to the cutoff used in that previous study because we found similar biphasic distribution in the extent of ADP-induced platelet aggregation in the two studies.

Results

We studied 195 patients, excluding 12 patients taking potentially active antiplatelet agents at baseline (aspirin and ticlopidine). After an average follow-up of 642 days (range, 18 to 1298 days), there were 25 acute CHD events (11 SD and 14 AMI), giving a average incidence rate of 7.31 events per year per hundred patients. The remaining 170 patients were considered free of acute CHD events. In the same period of time, 18 deaths not related to acute CHD occurred; 7 patients developed progressive graft failure without clinical evidence of acute rejection or CHD events; in these patients, autopsy findings suggested that extended coronary atherosclerosis was the cause of the graft failure, despite the absence of recent infarction. Causes of the other deaths were viral myocarditis (2), cancer (3), stroke (3), acute pancreatitis (1), acute leukemia (1), and suicide (1). Follow-up time of these 18 patients was included up to the date of their death or retransplantation. No patient was lost to follow-up.

The mean levels of platelet aggregation and lipids as well as drug treatment of the two groups (with and without acute CHD events) at baseline are depicted in Table 1. Drug treatment, including cyclosporine and steroids, was very similar in the two groups, whereas platelet aggregation tended to be higher and both HDL-C and LDL-C lower in the group with CHD events. Univariate associations between age, length of time since transplant, platelet aggregation, and lipid levels and subsequent CHD events were assessed with the Cox model (Table 2). The most potent predictors of acute CHD were the secondary wave of ADP-induced platelet aggregation (positive association) and total cholesterol (inverse correlation). Since both HDL-C and LDL-C (the sum of which is approximately total cholesterol) were individually also significantly associated with the risk of subsequent acute events, they were entered into the model instead of total cholesterol to assess their simultaneous effect as predictors. Only platelet aggregation (P=.001) and HDL-C (P=.03) remained independent predictors in multivariate analysis (Table 3). In clinical terms, it means that patients with baseline ADP-induced platelet aggregation (secondary wave) >35%, between 35% and 53%, or between 19% and 35% (corresponding to quartile distribution) had a risk of presenting acute CHD events 12, 10, or 3 times higher, respectively, than patients with platelet aggregation <19% (the lowest quartile). Similarly, patients with HDL-C >1.70 mmol/L, between 1.70 and 1.35 mmol/L, or between 1.35 and 1.15 mmol/L had a risk of presenting acute CHD events 4, 2.5, and 1.5 times lower, respectively, than patients with HDL-C <1.15 mmol/L (the lowest quartile). The ability of ADP-induced platelet aggregation (secondary wave) to separate patients into different risk strata is further illustrated by comparing “low” and “high” aggregation according to a subdivision based on a cutoff point of 36%, as described in a previous study in a large group of nontransplanted subjects. Indeed, in the Caerphilly study, there was a biphasic distribution of the extent of the secondary wave of ADP-induced platelet aggrega-
tion; 36% appeared to be the most suitable cutoff point to separate "high" and "low" aggregation groups. The distribution of ADP-induced platelet aggregation in the present study tended to be very similar to that of the Caerphilly study (data not shown). None of the clinical and biological characteristics of the two groups were different except the proportion of patients who developed SD or AMI (P=.0009) (Fig 1). The survival analysis shown in Fig 2 indicated that the relative risk of presenting SD or AMI was 4.3 times higher (95% confidence interval, 1.9 to 9.7; P=.0001) in the "high" PA group than in the "low" PA group.

Discussion

The present study demonstrates that platelet aggregation closely predicts acute CHD events in HT recipients. In other terms, if high platelet aggregation was present at baseline, there was a markedly enhanced risk of subsequent SD and fatal and nonfatal AMI in these patients. Low HDL-C also appears to be an independent risk factor of developing acute CHD events. Moreover, the present study confirms that SD and AMI are the main complications limiting long-term survival beyond the first 6 months after heart transplantation.

The pathogenesis of SD in these patients is unknown. Nevertheless, since we found a close relation between platelet reactivity and acute CHD, the cause of SD may have been transient thrombotic and/or spastic coronary occlusion. The sequence of ischemia and reperfusion is known to be a prevalent mechanism of malignant arrhythmias and SD. In addition, coronary spams have been repeatedly reported in HT recipients, possibly as a result of potent vasospastic substances (serotonin and thromboxane) released from activated platelets or of sympathetic reinnervation.

The results of the present study in HT recipients are concordant with those in nontransplanted subjects who die suddenly or develop AMI: increased spontaneous or ADP-induced platelet aggregation was also observed in these patients. This suggests a similar pathogenesis of SD and AMI in transplanted and nontransplanted subjects, possibly with a key role of platelets in both conditions.

Platelet hyperaggregability seems to be a common feature of HT recipients, independent of the disease responsible for the transplantation. The hypothesis that SD or AMI occurred as an acute thrombotic complication and not only as a terminal step of a slow progressive obstructive process is substantiated by the lack of correlation between the length of time since transplant and subsequent SD and AMI. In some subjects of the present study, SD and AMI occurred early after transplantation, at a time when the fibrotic concentric intimal thickening was probably not de-
TABLE 2. Univariate Associations Between Selected Variables Recorded at Baseline and Subsequent Acute CHD Events

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.034</td>
<td>0.991-1.078</td>
<td>.12</td>
</tr>
<tr>
<td>Length of time since transplant, mo</td>
<td>1.003</td>
<td>0.982-1.025</td>
<td>.78</td>
</tr>
<tr>
<td>Platelet aggregation, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombin-induced</td>
<td>1.086</td>
<td>1.011-1.167</td>
<td>.02</td>
</tr>
<tr>
<td>ADP-induced</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary wave</td>
<td>1.061</td>
<td>1.013-1.112</td>
<td>.01</td>
</tr>
<tr>
<td>Secondary wave</td>
<td>1.031</td>
<td>1.013-1.049</td>
<td>.0007</td>
</tr>
<tr>
<td>Lipid levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>0.707</td>
<td>0.547-0.914</td>
<td>.008</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.949</td>
<td>0.716-1.258</td>
<td>.71</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>0.764</td>
<td>0.605-0.964</td>
<td>.02</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.288</td>
<td>0.101-0.819</td>
<td>.01</td>
</tr>
<tr>
<td>Apoprotein B100, g/L</td>
<td>0.520</td>
<td>0.212-1.273</td>
<td>.15</td>
</tr>
<tr>
<td>Apoprotein A1, g/L</td>
<td>0.222</td>
<td>0.058-0.85</td>
<td>.02</td>
</tr>
</tbody>
</table>

RR indicates relative risk; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol.

TABLE 3. Multivariate Analysis (Cox Model Adjusted for Age and Length of Time Since Transplant)

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet aggregation, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ADP-induced, secondary wave)</td>
<td>1.035</td>
<td>1.013-1.057</td>
<td>.001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>0.756</td>
<td>0.562-1.018</td>
<td>.07</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.313</td>
<td>0.107-0.920</td>
<td>.03</td>
</tr>
</tbody>
</table>

RR indicates relative risk; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol.

Table 3 shows that the Cox proportional hazards regression analysis adjusted for age and length of time since transplant. The table lists the RR, 95% CI, and p-values for various variables, including platelet aggregation, LDL-C, and HDL-C. The RR indicates the relative risk of the event (in this case, acute CHD) for a one-unit increase in the variable, with the 95% CI providing a measure of the precision of the estimate.

Concerning the relation between low HDL-C and CHD, the usual explanation is that HDL-C is a mediator of reverse cholesterol transport. However, graft coronary arteries did not show massive lipid accumulation. Rather, organized thrombi, granulation tissue, and intimal concentric hyperplasia were the dominant features observed. Could HDL-C have other favorable effects on CHD in addition to cholesterol transport? It has been shown recently that apoprotein A1, the major protein component of HDL-C, is a prostacyclin-stabilizing factor. In addition, in patients with accelerated CHD, low HDL-C was associated with a reduction in the half-life of prostacyclin, the main endogenous antiplatelet and vasodilatory substance. The interest of this hypothesis is that we have recently described a drastic reduction in systemic prostacyclin production in HT recipients. Whether prostacyclin deficiency is related to cyclosporine, to antiendothelial antibody cytotoxicity, or to both factors needs to be further elucidated. Low HDL-C may nevertheless indicate that chronic immunosuppressive or low-grade subclinical immune injury enhances the production of reactive oxygen species, resulting in lipid peroxidation and platelet hyperreactivity.

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Table 2 shows that the Cox proportional hazards regression analysis adjusted for age and length of time since transplant. The table lists the RR, 95% CI, and p-values for various variables, including platelet aggregation, LDL-C, and HDL-C. The RR indicates the relative risk of the event (in this case, acute CHD) for a one-unit increase in the variable, with the 95% CI providing a measure of the precision of the estimate.

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Fig 2. Graph showing event-free survivals of patients with "low" or "high" platelet aggregation. The event-free survival rate was significantly higher (P<0.0001) in patients with "low" (<38%, solid line) ADP-induced platelet aggregation (secondary prevention).

rectly reflect a thrombotic and vasospastic tendency. This is in accordance with recent cross-sectional studies showing associations between graft CHD and prothrombotic abnormalities.33,34

In conclusion, the strong association between high platelet aggregation and subsequent SD and AMI in the present study supports the view that efficiently inhibiting platelet reactivity seems to be the logical therapeutic approach to be actively pursued in HT recipients. Unfortunately, aspirin, the usual antiplatelet drug, does not prevent graft CHD in animals6 and in humans,6 apparently because it does not inhibit platelet aggregation after transplantation.2 An alternative drug could be ticlopidine, as shown recently in preliminary studies.35

References

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_Circulation_. 1994;89:2590-2594
doi: 10.1161/01.CIR.89.6.2590

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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