Clinical and Population Studies

Coronary Artery Calcification in Hemophilia A
No Evidence for a Protective Effect of Factor VIII Deficiency on Atherosclerosis


Objective—Ischemic heart disease mortality is lower in hemophilia patients than in the general male population. As coagulation plays a role in the inflammatory pathways involved in atherogenesis, we investigated whether the clotting factor deficiency protects hemophilia patients from developing atherosclerosis.

Methods and Results—Coronary artery calcification, measured with multidetector-row computed tomography, was compared between 42 men, ≥59 years, with severe or moderate hemophilia A, and 613 nonhemophilic men from the Rotterdam Study, a prospective population-based study. None of the study subjects were HIV infected or had a history of cardiovascular disease. Coronary artery calcification was quantified by calculating the Agatston score and calcification mass. Data were analyzed using linear regression. Mean difference (β) of the natural log–transformed Agatston score between men with and without hemophilia was 0.141 (95% CI –0.602 to 0.885, P=0.709). Results did not change after adjustment for age, body mass index, hypercholesterolemia, hypertension, and use of antidiabetic medication (β=0.525, 95% CI –0.202 to 1.252, P=0.157). Comparable results were found for calcification mass.

Conclusion—The extent of coronary artery atherosclerosis is comparable between elderly men with and without hemophilia. Results from this study underline the importance of screening and treating atherosclerosis risk factors in hemophilia patients. (Arterioscler Thromb Vasc Biol. 2012;32:799-804.)

Key Words: atherosclerosis ■ coagulation ■ coronary heart disease ■ hemophilia ■ imaging

Since the introduction of clotting factor concentrates, life expectancy of hemophilia patients has increased and now approaches that of the general male population.1,2 Consequently, aging hemophilia patients not only experience medical issues associated with their congenital bleeding disorder but are increasingly confronted with age-related comorbidities, including ischemic cardiovascular disease (CVD).3,4 Interestingly, cohort studies reported a reduced mortality due to ischemic heart disease (IHD) in hemophilia patients as compared with the general male population.1,2

Several mechanisms explaining this observation have been considered.5 Rosendaal et al6 showed that differences in the prevalence of cardiovascular risk factors between men with and without hemophilia could not account for the lower IHD mortality in hemophilia patients. Another, very plausible, explanation could be that the hypocoagulable state of hemophilia patients has a protective effect on thrombus formation, which precipitates myocardial infarction.7,8 In this study, we focused on the hypothesis that hemophilia patients develop less atherosclerosis than men without hemophilia. Atherosclerosis is an inflammatory disease, which develops in response to injury of the endothelium.9,10 Current evidence supports considerable cross-talk between thrombosis and the inflammatory pathways involved in the different stages of atherogenesis (eg, plaque initiation and expansion, and fibrous cap degradation and rupture).11,12 Thrombin is the main effector of the coagulation cascade and links coagulation to inflammation by having proinflammatory effects, via protease-activated receptors, on the cells involved in atherogenesis.11,12 Activation of prothrombin into thrombin is less

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in hemophilia patients as compared with nonhemophilic subjects. This might reduce the stimulation of proinflammatory pathways supporting atherosclerosis. As compared with apolipoprotein E–deficient mice, apolipoprotein E and FVIII double-deficient mice developed considerably fewer early-stage atherosclerotic lesions, which were almost devoid of fibrinogen, fibrin and platelets. At a later stage, FVIII deficiency only delayed plaque progression. Recently, Fabri et al showed that FVIII deficiency was associated with an antiatherosclerotic phenotype in apolipoprotein E–deficient mice. However, FVIII deficiency did not influence the degree of atherosclerosis in mice lacking the low-density lipoprotein receptor, a model more resembling the human situation. Conflicting results have also been reported by studies comparing nonhemophilic subjects. This could be due to heterogeneous intima-media thickness (IMT) between hemophilia patients and the general male population.

Extended cohort) were invited to undergo an MDCT scan of the heart. Within a single breath hold, 3-mm-thick slices were acquired (scan parameters: 128×0.625-mm collimation, 120 kVp, 30–80 mAs [depending on weight], prospective ECG triggering in mid-diastole). Quantification of CAC was performed by a trained scan reader (A.R.), blinded to the clinical data, using software for calcium scoring (Heartbeat-CS, EBW, Philips Medical Systems, Best, the Netherlands). All voxels with an attenuation of ≥130 Hounsfield units were highlighted by the software. Atherosclerotic calcifications in the left main, the left anterior descending, the left circumflex, and the right coronary arteries were manually identified by the scan reader. For each calcified lesion, the Agatston score was calculated as the product of the area of a calcified lesion and a factor was assigned according to the maximum attenuation value of the lesion. The total Agatston score was calculated by adding up the scores of each individual lesion. Because of the limited interscan reproducibility of the Agatston score, an algorithm quantifying the calcification mass, with lower interscan variability, was introduced. Calculation mass (mg hydroxyapatite) was calculated as the volume of a calcification times the mean computed tomography number multiplied by a calibration factor c (mg/Hounsfield units×cm²).

Comparison data on CAC and cardiovascular risk factors were obtained from the Rotterdam Study, a prospective population-based study with the aim of assessing the occurrence of chronic diseases in an aging population and to clarify their determinants. In 1990, all inhabitants of a suburb of Rotterdam, the Netherlands, aged ≥55 years, were invited, and 7983 agreed to participate (78%). In 2000, the cohort was extended with 3011 subjects. From September 2003 until February 2006, all participants who completed a center visit during the study visit of hemophilia patients to the University Medical Center Utrecht, characteristics of hemophilia were assessed. In addition, information on medical history, family history of CVD, medication use, and smoking behavior was collected. In the Rotterdam Study, data collection was also collected. In both hemophilia patients and people included in the Rotterdam Study, systolic and diastolic blood pressures were measured at the right brachial artery in a sitting position. The mean of 2 consecutive measurements was used. Hypertension was defined as a systolic blood pressure of ≥140 mm Hg, a diastolic blood pressure of ≥90 mm Hg, or use of antihypertensive medication. Height and weight were measured, and body mass index was calculated as weight divided by height squared (kg/m²). Total cholesterol, high-density lipoprotein cholesterol, triglycerides, and glucose were measured in blood samples from both hemophilia patients and Rotterdam Study subjects. Low-density lipoprotein cholesterol was calculated using Friedewald's equation.

Hypcholesterolemia was defined as a total cholesterol level ≥6.5 mmol/L or use of lipid-lowering medication.

Ten-year risk of coronary heart disease (CHD) was calculated for all subjects, using the Framingham Point Scores as recommended in the third report of the National Cholesterol Education Program. Points were assigned to categorized risk factors and summed. The summary score corresponds to a 10-year predicted risk of CHD (nonfatal myocardial infarction or fatal CHD) and can be classified into low (<10%), intermediate (10% to 20%), and high (>20%) risk categories.

Hemophilia patients were scanned with a 128×0.625 detector and the Radiation Protection Unit of the Erasmus University Medical Center (Rotterdam, the Netherlands). All participants provided written informed consent.

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factor concentrate per year. Fourteen (33.3%) patients were treated on demand and used FVIII activity of 1% (Table 1). Most hemophilia patients were treated on demand and used <50 000 U of clotting factor concentrate per year. Fourteen (33.3%) patients were infected with hepatitis C, whereas 16 (38.1%) patients had been successfully treated or had spontaneously cleared the virus (Table 1). The selection procedure described earlier resulted in a comparison group of 613 nonhemophilic men.

Cardiovascular risk factors are described in Table 2. Mean age of the study population was 66.5 years (standard deviation 4.6). In both hemophilia patients and nonhemophilic men, age ranged from 59 to 77 years. As compared with men without hemophilia, more hemophilia patients smoked. Mean body mass index, mean total cholesterol levels, and the proportion of subjects with hypercholesterolemia were higher in the comparison group than in hemophilia patients. Of 42 hemophilia patients, 18 (42.9%) used antihypertensive medication and 32 (76.2%) were hypertensive, whereas only 26.1% of the comparison population used antihypertensive medication and 32 (76.2%) were hypertensive.

Results

Sixty eligible hemophilia patients were invited, and 42 agreed to participate (70%). Half of the hemophilia patients had a FVIII activity of <1% (Table 1). Most hemophilia patients were treated on demand and used <50 000 U of clotting factor concentrate per year. Fourteen (33.3%) patients were infected with hepatitis C, whereas 16 (38.1%) patients had been successfully treated or had spontaneously cleared the virus (Table 1). The selection procedure described earlier resulted in a comparison group of 613 nonhemophilic men.

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Agatston scores obtained in the Rotterdam Study could be reproduced by the software and scan reader at the University Medical Center Utrecht (Spearman’s rank correlation coefficient 0.986, P < 0.001). CAC outcomes are summarized in Table 3.
Table 3. Coronary Artery Calcification Outcomes

<table>
<thead>
<tr>
<th>Coronary Artery</th>
<th>Patients With Hemophilia (n=42)</th>
<th>Nonhemophilic Males (n=613)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agatston score</td>
<td>129.60 (17.28–380.78)</td>
<td>88.50 (9.35–382.95)</td>
</tr>
<tr>
<td>ln(Agatston score +1)</td>
<td>4.20±2.31</td>
<td>4.06±2.38</td>
</tr>
</tbody>
</table>

Categories of Agatston score

<table>
<thead>
<tr>
<th>Category</th>
<th>Patients With Hemophilia</th>
<th>Nonhemophilic Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–9</td>
<td>10 (23.8)</td>
<td>155 (25.3)</td>
</tr>
<tr>
<td>10–99</td>
<td>9 (21.4)</td>
<td>162 (26.4)</td>
</tr>
<tr>
<td>100–399</td>
<td>13 (31.0)</td>
<td>151 (24.6)</td>
</tr>
<tr>
<td>≥400</td>
<td>10 (23.8)</td>
<td>145 (23.7)</td>
</tr>
<tr>
<td>Agatston score ≥1000</td>
<td>5 (11.9)</td>
<td>60 (9.8)</td>
</tr>
<tr>
<td>Calcification mass</td>
<td>25.55 (2.85–71.98)</td>
<td>16.96 (2.35–70.65)</td>
</tr>
<tr>
<td>ln(calcification mass +1)</td>
<td>2.92±1.86</td>
<td>2.78±1.85</td>
</tr>
</tbody>
</table>

Values of Agatston score and calcification mass are expressed as median and interquartile range, respectively. ln indicates natural logarithm.

Table 4. Regression Coefficients Describing the Association Between the Natural Log–Transformed Agatston Score and Calcification Mass and Having Hemophilia

<table>
<thead>
<tr>
<th>Regression Coefficient</th>
<th>β (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ln(Agatston score +1):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>univariate model</td>
<td>0.141 (-0.602 to 0.885)</td>
<td>0.709</td>
</tr>
<tr>
<td>ln(Agatston score +1):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>multivariate model</td>
<td>0.525 (-0.202 to 1.252)</td>
<td>0.157</td>
</tr>
<tr>
<td>ln(calcification mass +1):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>univariate model</td>
<td>0.139 (-0.439 to 0.717)</td>
<td>0.637</td>
</tr>
<tr>
<td>ln(calcification mass +1):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>multivariate model</td>
<td>0.428 (-0.138 to 0.994)</td>
<td>0.138</td>
</tr>
</tbody>
</table>

Multivariate model: adjusted for age, body mass index, hypercholesterolemia, hypertension, and the use of antidiabetic medication. β indicates regression coefficient or mean difference; CI, confidence interval.

Discussion

In this study, we found no evidence for a protective effect of congenital FVIII deficiency on the development of CAC measured with MDCT.

Previous studies, using B-mode ultrasonography to compare carotid and femoral IMT between hemophilia patients and nonhemophilic subjects, reported conflicting results.17–21 Srženec et al21 and Sartori et al20 found no differences in IMT, whereas 3 other studies17–19 did show a lower IMT, fewer atherosclerotic plaques, or both in hemophilia patients. In these studies, patients were relatively young; mean age ranged from 40 to 58 years. Possibly, atherosclerotic burden of the study population was so low that it complicated the detection of differences between the groups. A major disadvantage in most studies is the heterogeneous study population, combining patients with severe, moderate, and mild hemophilia A or B or von Willebrand disease.17,18,20,21 Clotting factors VIII and IX and von Willebrand factor play different roles in the development of atherosclerosis.12,34 Combining these disorders in 1 study might result in masking a possible relationship between 1 of the disorders and atherosclerosis. Unfortunately, sample sizes are too small for valid subgroup analyses. Moreover, comparison groups were not well chosen and, in some, not well described.17–21 In addition, confounding was not accounted for in the analyses. These factors make interpretation of the results difficult.

In the current study, only patients with a residual FVIII activity ≤5% were included. If a protective effect of FVIII deficiency on the development of atherosclerosis exists, it can be expected to be most pronounced in patients with severe or moderate hemophilia A. The hemophilia study population had a mean age of 65 years, thereby having a high probability of having developed atherosclerosis. We excluded HIV-positive patients because most of them are treated with highly active antiretroviral therapy, which influences CVD risk.35 A well-defined, large sample from the Dutch general male population was used for comparison.

Hemophilia patients were more often hypertensive as compared with men without hemophilia. This result is in accordance with other studies.6,36–39 However, the underlying mechanism of this observation is unknown. Mean total cholesterol levels and the proportion of subjects with hypercholesterolemia were lower in the hemophilia patients than in the comparison group, which is consistent with the results of Rosendaal et al6. This could be explained by the association between chronic hepatitis C infection and a favorable lipoprotein profile.40 We calculated the 10-year CHD risk, using the Framingham Point Scores, and found no differences in risk category distributions between men with and without hemophilia.26 This prediction algorithm is based on and developed for the general population.31 Consequently, true risks for the hemophilia study population will be different from the calculated risks. However, to compare a summary of the prevalence of cardiovascular risk factors between hemophilia patients and the general male population, in a scientific
setting, the Framingham Point Scores can be used. The role of the available cardiovascular risk prediction algorithms in hemophilia patients in a clinical setting is less clear.

The lack of evidence for a lower extent of coronary artery atherosclerosis in hemophilia patients makes that alternative hypotheses, explaining the reduced IHD mortality in these patients, should be considered or reconsidered. In 1990, Rosendaal et al. concluded that only a fraction of the reduced IHD mortality in hemophilia patients as compared with the general male population could be explained by differences in risk factor prevalence. Since then, the elderly hemophilia patient population has expanded, and competing risks due to HIV and hepatitis C infection are now less important. Therefore, large prospective studies are needed to reassess the current prevalence of risk factors and their influence on CVD risk in hemophilia patients. As mentioned before, a very plausible explanation would be that the hypocoagulable state of hemophilia patients has a protective effect on thrombus formation. Possibly, hemophilia patients more often develop a mural thrombus on plaque rupture than a fatal occlusive thrombus as compared with nonhemophilic males. As several cohort studies investigated IHD death in hemophilia patients, only 1 study compared IHD prevalence between men with and without hemophilia. The authors found that the rate of IHD hospital discharges among hemophilia patients 65 years and older was nearly 30% lower than that of nonhemophilic males. This might imply that hemophilia patients experience plaque rupture less often or that they have a “silent” plaque rupture more often than nonhemophilic males do. When plaque rupture or erosion is silent and thrombosis does not lead to arterial occlusion, platelets and the thrombotic response are important in the process of plaque progression. Degranulating platelets release platelet-derived growth factor and transforming growth factor-β, which cause a fibrotic healing response, leading to increased collagen accumulation, smooth muscle cell proliferation and accelerated progressive luminal narrowing. This might explain the comparable amount of atherosclerosis found in the current study. The risk of plaque rupture mainly depends on plaque composition. Vulnerable plaques have thin or eroded fibrous caps that overlay large lipid cores and contain a lot of inflammatory cells. Hemophilia patients might develop less vulnerable plaques. Plaque composition can be investigated with magnetic resonance imaging. In addition, markers of plaque vulnerability, such as matrix metalloproteinases and fibrin, can be targeted with antibodies and visualized with molecular magnetic resonance imaging.

Some limitations of this study need to be discussed. Only 42 patients with hemophilia A were included, which may have affected the results. However, no tendency for a difference in the extent of CAC between men with and without hemophilia was observed. Therefore, we think it is unlikely that larger numbers would have changed the results. Hemophilia patients receive prophylactic or on-demand treatment with clotting factor concentrates. If a protective effect of FVIII deficiency exists, it could have been influenced by this treatment. However, patients with severe hemophilia temporarily become mild hemophiliacs after prophylactic treatment, and FVIII activity decreases to ≤5% before the next prophylactic treatment in many of them. Thus, FVIII activity is not normalized. In addition, during a large part of the lives of elderly hemophilia patients, treatment with clotting factor concentrates was not (regularly) available. On the other hand, the hemophilia patient who is treated for its disease is the patient we see in our clinic every day, and the cohort studies showing a lower IHD mortality are based on this population. It is not clear whether hepatitis C infection is positively related to atherosclerosis, as studies investigating this relation by measuring IMT reported conflicting results. Therefore, patients with past or current hepatitis C infection were not excluded. Different MDCT scanners were used to assess CAC in men with and without hemophilia, and different scan readers performed CAC quantification. However, a standard calcium-scoring acquisition protocol with 3-mm-thick slices and prospective ECG triggering was used in both groups. In addition, the quantification process has been largely automated, algorithms for calculating the Agatston score and calcification mass have been standardized, and quantification was shown to be reproducible between centers.

In conclusion, the amount of coronary artery atherosclerosis is comparable between elderly patients with hemophilia A and men from the general population. Despite the relative protection against IHD mortality, the incidence of ischemic CVD in hemophilia patients is increasing as life expectancy now approaches that of the general population. Treatment of these patients is complex, because of the delicate equilibrium between bleeding and thrombosis, and evidence-based treatment guidelines are lacking. Results from this study underline the importance of screening and treating atherosclerosis risk factors to minimize the risk of cardiovascular events in hemophilia patients.

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Disclosures

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