Diabetes Duration Is Associated With Increased Thin-Cap Fibroatheroma Detected by Intravascular Ultrasound With Virtual Histology

Jason B. Lindsey, MD; John A. House, MS; Kevin F. Kennedy, MS; Steven P. Marso, MD

**Background**—Coronary plaque classified as thin-cap fibroatheroma (TCFA) is believed to be associated with plaque rupture and coronary heart disease–related events. Although an association between duration of diabetes and increased coronary heart disease risk has been demonstrated, the relationship between TCFA and diabetes duration is unknown.

**Methods and Results**—Prospective registry of diabetic patients undergoing diagnostic coronary angiography and intravascular ultrasound (IVUS) enrolled in a diabetic gene and biomarker banking registry. Plaque composition in the most diseased 10-mm segment of a single coronary artery was assessed using IVUS virtual histology and was classified by phenotype as IVUS-defined adaptive intimal thickening, pathological intimal thickening, TCFA, fibroatheroma, or fibrocalcific. Patients (n=54) were stratified by duration of diabetes (<10 or ≥10 years). Patients with diabetes ≥10 years were older, less likely to have a history of tobacco use, had higher total cholesterol levels, and were more likely to be treated with insulin compared with patients with diabetes <10 years. Longer duration of diabetes was associated with greater plaque burden in the most diseased 10-mm segment (60.4% [53.4% to 66.8%] versus 50.2% [47.7% to 58.4%], P=0.008). The proportion of IVUS-defined TCFA in the ≥10-year group was greater than the <10-year group (54.4% [11.6% to 77.5%] versus 10.8% [0.0% to 26.1%], P=0.009). This association persisted after adjustment for multiple comparisons, clinical characteristics, and diabetes treatment.

**Conclusions**—In this cohort, longer duration of diabetes was associated with IVUS-defined TCFA, a plaque phenotype associated with risk of rupture and coronary heart disease events.

**Clinical Trial Registration**—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00428961. (Circ Cardiovasc Intervent. 2009;2:00-00.)

Key Words: diabetes mellitus ■ atherosclerosis ■ imaging ■ plaque

Patients with diabetes mellitus are at heightened risk for premature mortality and nonfatal myocardial infarction. Diabetes mellitus is recognized as a coronary heart disease (CHD) risk equivalent by National Cholesterol Education Program Adult Treatment Panel III guidelines. The etiology of the increased risk for myocardial infarction and cardiovascular death in those with diabetes is not fully understood.

<table>
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<th>Clinical Perspective on p ●●●</th>
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| Diabetes duration is thought to contribute to excess CHD risk. Several studies have investigated the association between diabetes duration and CHD risk. In diabetic women with and without existing CHD, death caused by CHD has been shown to increase with duration of diabetes. An independent association between fatal CHD and increasing duration of diabetes has also been reported in men. Framingham Heart Study investigators reported a 1.8-fold increase in risk of CHD death and 1.4-fold increase in risk of CHD for every 10-year increase in diabetes duration. Furthermore, necropsy data suggest that duration of diabetes is associated with more extensive coronary atherosclerotic lesions. Others have studied the role of high-risk coronary plaque resulting in rupture and increased risk for CHD and cardiovascular death in diabetic patients. Characterized by a predominant necrotic core, intraplaque hemorrhage, calcification, and a thin layer of connective tissue, these vulnerable plaques that result in myocardial infarction and sudden death are termed thin-cap fibroatheroma (TCFA). By using intravascular ultrasound with virtual histology (IVUS-VH), TCFA has been demonstrated to be more common in plaques of acute coronary syndromes compared with stable angina patients. In another IVUS-VH study, individuals with diabetes had a greater prevalence of necrotic core and IVUS-defined (ID) TCFA than their nondiabetic counterparts. Although a causal relationship between ID-TCFA and adverse cardiovascular outcomes has yet to be established, the finding that diabetic subjects have an increased prevalence of ID-TCFA suggests another pathobi-
logic mechanism potentially contributing to their increased CHD and cardiovascular risk.

Because the relationship between duration of diabetes and ID-T DFA is unknown, the objective of this study was to describe the proportion of ID-T DFA by diabetes duration in a population of diabetic patients undergoing coronary angiography.

Methods

Study Population

The Diabetes Genome Project was a prospective registry. Details have been published. Analysis of the medial-adventitial and luminal borders for each analyzable cross-sectional frame for the entire pullback length was performed by IVUS core laboratory personnel. Software was used to reconstruct IVUS B-mode images from the radiofrequency data (pcVH version 2.2, Volcano) and to calculate geometric and composition data for each IVUS frame. Composition data were color-coded as follows: fibrous (green), fibrofatty (light-green), dense calcium (white), and necrotic core (red). Compared to histopathology, this software has a predictive accuracy of 94% for fibrous, 94% for fibrofatty, 96% for necrotic core, and 97% for dense calcium. Qualitative IVUS analysis was performed according to American College of Cardiology guidelines.14

Using IVUS-VH composition data, an automated pixel detection algorithm (Volcano Corp) assigned a phenotype to each IVUS frame. This methodology is based on a histopathologic classification system8 (Figure). Confluence for each compositional element was set at a minimal area for this study. A confluent area was defined as a circular area represented by continuous composition of the same tissue type for a minimum diameter of 12 pixels or 0.08 mm² on a 400×400 VH-IVUS image. Atherosclerotic plaque consisted of plaque >600 µm thick was assigned to 1 of 5 phenotypes as follows (1) ID adaptive intimal thickening: any plaque <600 µm; (2) ID pathological intimal thickening: predominately fibrous tissue with or without >15% fibrofatty tissue and without either confluent necrotic core or confluent dense calcium; (3) ID fibrocalcific: confluent dense calcium without confluent necrotic core; (4) ID fibroatheroma: confluent necrotic core not at the lumen or, if at the lumen surface, then not exceeding 14 pixels along the circumference of the lumen or if, at the lumen surface, then not exceeding 14 pixels along the circumference of the lumen on 3 consecutive frames with or without confluent dense calcium.

Intravascular Ultrasound

Patients enrolled in the IVUS substudy underwent IVUS at the discretion of the operator for the following indications: (1) assessment of an indeterminate lesion; (2) investigation of a culprit lesion; (3) clarification of coronary lesion extent. Patients younger than 18 years with hemoglobin A1c, demographic, anatomic, and biochemical laboratory measures were collected on each patient. DNA was also stored for future analysis. Enrollment is complete and consists of 1607 patients. The current report is a subset of the population who underwent IVUS-VH. This study was approved by the Saint Luke’s Hospital institutional review board.

Diabetes Classification

For this substudy, an established clinical diagnosis of diabetes was required for inclusion. Patients with diabetes were treated pharmacologically or by diet. Diabetes duration was determined by self-report. Patients were stratified a priori by diabetes duration <10 years or ≥10 years based on previous work2–4 demonstrating an association between diabetes duration and cardiovascular events.

Statistical Analysis

Data were dichotomized by presence of diagnosed diabetes <10-year or ≥10-year duration, with continuous data represented as
mean ± SD or median (interquartile range), and categorical data represented by percent. Demographic data were analyzed using student t tests, Wilcoxon rank-sum tests, or χ² as appropriate. Grayscale IVUS and ID phenotypes are represented as median (interquartile range). The end points of interest were the 5 continuous ID phenotypes. Comparisons of the ID phenotypes by diabetes duration were performed using Wilcoxon rank-sum tests. Because there were 5 end points of interest, a Bonferroni-Holm multiple comparisons adjustment for the 5 ID phenotypes was performed on the Wilcoxon rank-sum test results. Only the ID-TCFA phenotype held significance after the multiple comparisons adjustment. On graphical representation of ID-TCFA, data were nonnormally distributed and right skewed. In models used to establish an appropriate distribution, ID-TCFA values that were zero were assigned an arbitrary value of 0.05%. It was determined that ID-TCFA was consistent with an exponential distribution (Kolmogorov-Smirnov goodness of fit P>0.15). To determine the difference in ID-TCFA between the two diabetes duration groups, a generalized linear model was developed adjusting for age, sex, lipid status, acute coronary syndrome on presentation, smoking, congestive heart failure, and baseline hemoglobin A1c values with the result reported as the difference in the estimated regression coefficient and corresponding standard error. Additional sensitivity models were developed by adding diabetes treatment to the above model. Statistical significance was defined as a 2-sided probability value of ≤0.05. Analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

Results
Baseline characteristics of study participants (n=54) are shown in Table 1. Median (interquartile range) duration of diabetes was 6.0 (3.0 to 8.0) years in the <10-year group and

| Table 1. Baseline Characteristics of Subjects by Duration of Diabetes Mellitus |
|-------------------------------|-------------------|-------------------|
| Diabetes <10 y (n=26) | Diabetes ≥10 y (n=28) | P     |
| Age, y | 58.5±9.2 | 65.8±10.0 | 0.007 |
| Male | 73.1 | 60.7 | 0.34 |
| White race | 88.5 | 92.9 | 0.66 |
| Body mass index | 31.0 (28.6 to 35.4) | 31.8 (27.7 to 34.4) | 0.82 |
| Hypertension | 96.2 | 100.0 | 0.48 |
| Hypercholesterolemia | 100.0 | 85.7 | 0.11 |
| Previous tobacco use | 73.1 | 32.1 | 0.003 |
| Heart failure | 34.6 | 17.9 | 0.16 |
| Previous myocardial infarction | 34.6 | 28.6 | 0.63 |
| Previous coronary artery bypass surgery | 11.5 | 10.7 | 0.99 |
| Previous percutaneous coronary intervention | 50.0 | 53.6 | 0.79 |

Clinical presentation
Stable angina or silent ischemia | 76.9 | 78.6 | 0.20 |
Acute coronary syndrome | 23.1 | 21.4 | 0.88 |

Hemoglobin A1c (%) | 7.1 (6.2 to 8.4) | 7.2 (6.8 to 8.3) | 0.92 |

IVUS Vessel (%) | 0.15 |
Left main coronary artery | 0.0 | 3.6 |
Left anterior descending coronary artery | 61.5 | 35.7 |
Left circumflex coronary artery | 23.1 | 25.0 |
Right coronary artery | 15.4 | 35.7 |
Multivessel coronary disease | 80.8 | 89.3 | 0.46 |
Multivessel PCI | 19.2 | 17.9 | 0.99 |
PCI during index procedure | 76.9 | 78.6 | 0.88 |

Lipoproteins
Total cholesterol | 142 (120 to 156) | 160 (143 to 175) | 0.05 |
LDL | 77 (55 to 90) | 86.5 (77 to 99) | 0.13 |
HDL | 33 (29 to 39) | 35 (32 to 50) | 0.12 |
Triglycerides | 139.5 (116 to 200) | 145 (84 to 208) | 0.87 |

Diabetes treatment
Insulin | 7.7 | 50 |
Oral medication | 73.1 | 50 |
Diet and exercise only | 19.2 | 0 |
Other medications
Statin | 69.2 | 75.0 | 0.64 |
Aspirin | 100.0 | 100.0 | 0.99 |

Values are mean ± SD, median (interquartile range), or %. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; PCI, percutaneous coronary intervention.
15.5 (12.0 to 21.5) years in the ≥10-year group. Patients with diabetes ≥10 years were older, less likely to have a history of tobacco use, had higher total cholesterol levels, and were more likely to be treated with insulin.

Table 2 depicts the standard grayscale and IVUS-VH measures of atheroma in the most diseased 10-mm segment. Total plaque volume was numerically greater, although not significant, in patients with diabetes duration ≥10 years. These nonsignificant numeric differences in plaque and vessel volume translated into a significant difference between groups in median plaque burden at the most diseased 10-mm segment (Table 2).

Table 2 also describes the proportion of the 5 plaque phenotypes as assessed by IVUS-VH and stratified by diabetes duration. The proportion of ID-TCFA in the ≥10-year group was 5-fold greater than that in the <10-year group (54.4% [11.6% to 77.5%] versus 10.8% [0.0% to 26.1%], P=0.009).

After adjustment for age, sex, lipid status, acute coronary syndrome presentation, smoking, congestive heart failure, and baseline hemoglobin A1c values, the relationship between ID-TCFA and diabetes duration persisted, with the amount of ID-TCFA in the <10-year group less than that in the ≥10-year group (−33.64±5.10%, P=0.019). In a sensitivity analysis, the association between ID-TCFA and diabetes duration persisted when diabetes treatment was added as a covariate (P=0.028).

Discussion

In a cohort of diabetic patients undergoing IVUS, increasing duration of diabetes was associated with greater plaque burden and ID-TCFA, a vulnerable plaque phenotype believed to lead to acute coronary events. Specifically, patients with diabetes ≥10 years had 5 times more ID-TCFA plaque than patients with diabetes <10 years. This finding persisted after adjustment.

The presence of diabetes is recognized as a CHD risk equivalent. Evidence is also accumulating that duration of diabetes is associated with greater risk of CHD events. An association between duration of diabetes and advanced atherosclerotic lesion formation has also been recognized.

Longer duration of diabetes is also associated with many untoward pathophysiologic effects, including microalbuminuria, nephropathy, and endothelial dysfunction, all associated with adverse cardiovascular outcomes. Patients with diabetes develop fewer coronary collateral vessels, have lower graft patency rates after coronary artery bypass surgery, and have impaired endothelial dysfunction. These and other mechanisms have been suggested to explain the increased incidence of CHD events in diabetic patients.

Atherosclerosis begins with plaque initiation as nonatherosclerotic deposits (intimal xanthoma) gradually progressing over time through higher risk subtypes (pathological intimal thickening, fibroatheroma, and TCFA). Thus, prolonged exposure to diabetes may contribute to an increased likelihood of cardiovascular events.

Many studies have shown that duration of diabetes is associated with cardiovascular events; others have not. Framingham Heart Study investigators demonstrated that duration of diabetes is associated with hard cardiovascular end points, including myocardial infarction and CHD death; for each 10-year increase in duration of diabetes mellitus, the adjusted hazard ratio for CHD was 1.38 and 1.86 for CHD death. In the Verona Diabetes Study, the investigators found that longer duration of diabetes was associated with an increase in the risk for all-cause and cardiovascular mortality. There was a monotonic increase in death rates with each 5-year incremental increase in diabetes duration. Further evidence linking diabetes duration and fatal CHD from the Nurses’ Health Study demonstrates that increasing diabetes duration was associated with a substantial increased risk for CHD death even after adjustment for possible confounders, including age. Thus, the preponderance of existing data supports an independent association between duration of diabetes and increased risk for CHD events and cardiovascular death. In fact, Nasu et al have shown that diabetes was associated with an increased prevalence of necrotic core and ID-TCFA. Our findings extend these by suggesting that duration, in addition to presence of diabetes, contributes to higher-risk atherosclerotic phenotypes such as ID-TCFA.

The link between TCFA, myocardial infarction, and cardiovascular death comes largely from autopsy data. In sudden

Table 2. Grayscale and IVUS-VH Analysis of the Most Diseased 10-mm Segment

<table>
<thead>
<tr>
<th>IVUS-VH phenotypes, %</th>
<th>Diabetes &lt;10 y (n=26)</th>
<th>Diabetes ≥10 y (n=28)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grayscale IVUS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen volume, mm³</td>
<td>77.8 (66.2 to 119.8)</td>
<td>68.5 (55.5 to 85.9)</td>
<td>0.111</td>
</tr>
<tr>
<td>Vessel volume, mm³</td>
<td>182.8 (143.1 to 227.2)</td>
<td>175.7 (142.4 to 221.4)</td>
<td>0.612</td>
</tr>
<tr>
<td>Plaque + media volume, mm³</td>
<td>100.3 (73.6 to 110.9)</td>
<td>115.5 (83.1 to 139.4)</td>
<td>0.269</td>
</tr>
<tr>
<td>Plaque burden, %</td>
<td>50.2 (47.7 to 58.4)</td>
<td>60.4 (53.4 to 66.8)</td>
<td>0.008</td>
</tr>
<tr>
<td>IVUS-VH phenotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adaptive intimal thickening</td>
<td>0.0 (0.0 to 3.1)</td>
<td>0.0 (0.0 to 0.0)</td>
<td>0.79*</td>
</tr>
<tr>
<td>Pathological intimal thickening</td>
<td>6.3 (0.0 to 43.5)</td>
<td>0.0 (0.0 to 21.9)</td>
<td>0.87*</td>
</tr>
<tr>
<td>Fibroatheroma</td>
<td>34.8 (10.0 to 46.9)</td>
<td>18.7 (5.4 to 30.6)</td>
<td>0.44*</td>
</tr>
<tr>
<td>Fibrocalcific</td>
<td>0.0 (0.0 to 7.4)</td>
<td>0.0 (0.0 to 5.4)</td>
<td>0.97*</td>
</tr>
<tr>
<td>Thin-cap fibroatheroma</td>
<td>10.8 (0.0 to 26.1)</td>
<td>54.4 (11.6 to 77.5)</td>
<td>0.009*</td>
</tr>
</tbody>
</table>

*P value adjusted for multiple comparisons.
cardiac death subjects, histologic examination of coronary arteries revealed a higher proportion of plaque burden and necrotic core in diabetic compared with nondiabetic subjects, partially suggesting that plaque progression and destabilization in diabetic subjects is more often mediated by a higher prevalence of necrotic core. Further work by Moreno et al\textsuperscript{9} compared diabetic and nondiabetic atherectomy specimens and found significantly more lipid-rich atheroma in the diabetic specimens. These findings suggest that diabetic plaque characteristics have increased vulnerability for coronary thrombosis and subsequent cardiovascular death.

**IVUS-VH Validation**

Tissue characterization using IVUS imaging platforms has recently been described.\textsuperscript{28} Several studies have validated IVUS-VH in evaluating tissue composition.\textsuperscript{19,29} Nair et al evaluated 899 regions of interest, including 184 plaques from 51 human coronary arteries. The overall predictive accuracy of IVUS-VH compared with histopathology was 94\% for fibrous tissue, 94\% for fibrofatty, 96\% for necrotic core, and 97\% for calcium. In this study, we describe an IVUS-VH classification system for phenotyping lesions. This system is derived from IVUS-VH compositional data and modeled after a simplified histopathologic classification scheme developed by Virmani et al.\textsuperscript{9} We categorized each IVUS frame into 1 of 5 phenotypes (adaptive intimal thickening, pathological intimal thickening, TCFA, fibroatheroma, fibrocalcific; Figure 1). We used an automated pixel detection algorithm to discriminate between these phenotypes. Intrasubject and interobserver variability are not present in assignment of frame phenotypes using this software algorithm but is rather solely attributable to variances in manual border detection (lumen and external elastic membrane cross-sectional area). Recently, Nasu et al\textsuperscript{11} used IVUS-VH to show that the presence of diabetes was associated with an increased prevalence of necrotic core and ID-TCFA. Our findings extend these by suggesting that duration, in addition to presence of diabetes, contributes to high-risk atherosclerotic phenotypes such as ID-TCFA.

**Limitations**

Duration of diabetes was determined by self-report and is subject to recall bias. However, others\textsuperscript{2,3,30} have used self-reported information to ascertain the presence of risk factors, including diabetes duration. Furthermore, the use of self-reported definitions for chronic diseases such as diabetes has been shown to be acceptable with accurate recall by patients with certain well-defined chronic diseases. Our sample size was small; however, this limitation is tempered by the large effect seen between ID-TCFA and diabetes duration and the well-phenotyped nature of the population of interest. The potential for survivor bias limits our findings. It is possible that persons died in the $<$10 year group. If high-risk plaque phenotypes were present in these individuals, then this would lead to a bias in our findings. However, this effect cannot be determined in this cross-sectional study design. No universal standardized definition of ID-TCFA exists; however, our definition was based on knowledge of TCFA derived from histopathologic studies. Finally, TCFA has not been linked to cardiovascular outcomes, and this study was not powered to elucidate such an association.

**Conclusions**

In a cohort of patients undergoing coronary angiography and IVUS, we demonstrate an association between duration of diabetes and increased prevalence of ID-TCFA, a high-risk coronary plaque phenotype.

**Acknowledgments**

We thank Jose Aceituno and Joseph Murphy for publication assistance.

**Source of Funding**

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**Disclosures**

Dr Marso is a consultant for Abbott Vascular, Volcano Corp, and Novo Nordisk and has received research grants from Volcano Corp, Amylin Pharmaceuticals, the Medicines Company, and Boston Scientific.

**References**

Patients with diabetes are at especially high risk for early cardiovascular death and nonfatal myocardial infarction. Duration of diabetes is thought to be an important determinant of cardiovascular risk for diabetic patients. In this study, we set out to determine whether duration of diabetes was associated with the frequency of a vulnerable plaque phenotype termed “thin-cap fibroatheroma,” as identified by intravascular ultrasound with virtual histology. Thin-cap fibroatheroma is believed to be highly prone to risk of plaque rupture. We performed intravascular ultrasound with virtual histology in 54 patients with diabetes who were classified by duration of diabetes, either less than or greater than 10 years. Patients with diabetes >10 years had more plaque overall as well as plaque classified as thin-cap fibroatheroma by intravascular ultrasound with virtual histology than patients who had diabetes <10 years. This relationship persisted when patient characteristics, risk factors, and drug therapy for diabetes were taken into account. This intravascular ultrasound study suggests that there is an association between diabetes duration and the frequency of vulnerable plaque.
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