In vivo virtual histology intravascular ultrasound correlates of risk factors for sudden coronary death in men: results from the prospective, multi-centre virtual histology intravascular ultrasound registry

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Aims
We hypothesized a relationship between virtual histology intravascular ultrasound (VH-IVUS) findings and risk factors histopathologically associated with sudden coronary death (SCD) in men: cigarette smoking and an increased total cholesterol-to-high-density lipoprotein cholesterol (TC/HDL) ratio (TC/HDL > 5).

Methods and results
We assessed volumetric VH-IVUS parameters in a consecutive series of 473 male patients: fibrous, fibro-fatty, dense calcium (DC), necrotic core (NC), and a calculated NC/DC ratio. Patients’ age was 61 ± 11 years, with 27% smokers and 69% having a lipid disorder. The NC/DC ratio was the only VH-IVUS parameter related to both TC/HDL ratio (r = 0.18, P = 0.0008) and low-density lipoprotein cholesterol levels (r = 0.17, P = 0.002); had a negative correlation with HDL-C levels (r = -0.11, P = 0.03); and was higher for smokers [median 1.98 (1.35–3.18)] vs. non-smokers [median 1.70 (1.23–2.53), P = 0.006]. An NC/DC value > 3 was the threshold that best identified smokers and/or patients presenting TC/HDL > 5 (odds ratio 3.0, 95% CI 1.7–4.9, P = 0.0001), and receiver-operator curves showed the superiority of the NC/DC ratio [area under curve (AUC) 0.64, P < 0.0001] over %DC (AUC 0.58, P = 0.006) or %NC (AUC 0.51, P = 0.43) to identify these patients.

Conclusion
The ratio of NC to calcification detected by VH-IVUS in diseased coronary segments is related to known risk factors for SCD and, thus, may be associated with a worse prognosis.

Keywords
Sudden death • Vulnerable plaque • Ultrasonics • Calcium • Necrotic core • Virtual histology

Introduction
Cigarette smoking impacts all phases of atherosclerosis, from endothelial dysfunction to acute clinical events, increasing the incidence of myocardial infarction (MI) and fatal coronary artery disease (CAD).1 Among the modifiable cardiac risk factors, smoking is particularly related to premature CAD2 and, in epidemiological studies, is consistently associated with an increased risk for unexpected sudden coronary death (SCD).3,4

Similar to smoking, hypercholesterolaemia is also associated with a diagnosis of premature atherosclerosis5 and has a positive relationship with increased CAD-related mortality.5 Furthermore, the benefits of lipid-lowering therapy in reducing acute MI and coronary death are now unquestionable.6

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Histopathological data obtained from male subjects after SCD showed that abnormal cholesterol levels—particularly elevated ratios of total cholesterol (TC) to high-density lipoprotein (HDL) cholesterol (TC/HDL)—are independently associated with plaque rupture as well as with the numbers of vulnerable plaques, whereas smoking predisposes patients to coronary thrombosis.7 Traditional coronary imaging modalities including angiography and intravascular ultrasound (IVUS) provide limited information regarding atherosclerotic plaque characteristics.8–14 Virtual histology (VH)-IVUS allows spectral analysis of backscatter-tered radiofrequency (RF) ultrasound signal; it has an 80–92% ex vivo and 87–92% in vivo accuracy for characterization of four basic plaque components: fibrous (Fi), fibro-fatty (FF), dense calcium (DC), and a lipid-rich necrotic core (NC).15,16 We hypothesized an in vivo relationship between VH-IVUS plaque characterization and known risk factors that have been linked pathologically to SCD in men.

Methods

Study protocol and definitions
Between August 2004 and July 2006, 990 patients in 42 interventional cardiology centres in the USA, Europe, and Japan were enrolled in the prospective, multi-centre, non-randomized, global VH-IVUS registry. The aim of the registry was to determine the clinical and laboratory correlates of VH-IVUS parameters in vivo in a consecutive, non-selected population of patients with age ≥18 years old. From this registry, we identified 473 male patients with a de novo culprit coronary lesion that could be crossed with an IVUS catheter, who were studied with either diagnostic or pre-interventional VH-IVUS. The Ethics Committee at each participating institution approved the protocol, and written informed consent was obtained from all patients.

A lipid disorder was defined as TC level ≥200 mg/dL, low-density lipoprotein cholesterol (LDL-C) ≥100 mg/dL, HDL-C <50 mg/dL, triglycerides ≥150 mg/dL, or medication use. Smoking was defined as current smoking or smoking stopped within 1 month prior to enrolment in the registry. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or use of an antihypertensive drug. Patients with diabetes mellitus had a confirmed diagnosis or were using antidiabetic medications (insulin or oral hypoglycaemic) at study entry. A risk profile for SCD was defined as TC/HDL ratio >5 and/or smoking on the basis of previous histopathological observations.7

Virtual histology intravascular ultrasound imaging protocol and data analysis
A phased-array, 20 MHz, 3.2 Fr IVUS catheter (Eagle Eye, Volcano Corporation, Rancho Cordova, CA, USA) was placed at a branch distal to the culprit lesion, and a motorized pull-back through the diseased segment was performed at 0.5 cm/s to a point proximal to the lesion site. During pull-back, grey-scale IVUS was recorded, raw RF data were captured at the top of the R-wave, and a colour-coded VH-IVUS map was generated (In-Vision Gold, Volcano Corporation, Rancho Cordova, CA, USA). Off-line volumetric reconstruction of the four tissue types along the whole pull-back was performed using pC Vaughan 2.1 software (Volcano Corporation). The four VH-IVUS plaque components were measured in every recorded frame and expressed as mean analysis segment cross-sectional area (CSA), absolute volume, and percentages of total plaque volume.15 On the basis of previous reports that unstable and/or ruptured plaques had larger NC content17 and less calcium when compared with stable and non-ruptured plaques,18–20 we calculated an NC/DC ratio in an attempt to identify patients with the same coronary risk profile as SCD male subjects.

Statistical analysis
Statistical analysis was performed with StatView 5.0.1 (SAS Institute, Cary, NC, USA). Categorical data were expressed as numbers or frequencies and compared using χ2 statistics. Continuous data were reported as mean ± SD or as median and interquartile range (IQR) according to its distribution. Unpaired Student’s t-test or one-way ANOVA test was used to test differences between two or more sets of data with normal distribution. If normality tests failed, the Mann–Whitney U statistic or Kruskall–Wallis test was performed. Linear regression or Spearman rank correlation was used to determine relationships between continuous variables. Multiple logistic regression was used to identify VH-IVUS predictors of a risk profile for SCD, and sensitivity and specificity curves were used to identify the optimal threshold for independent predictors, defined as the cutoff that separated patients with TC/HDL >5 and smokers with greatest accuracy. Logistic regression was performed to determine the odds ratio for all cutoff values tested, and the 95% confidence interval related to each odds ratio value was determined. A two-sided probability value <0.05 was considered statistically significant.

Results

Baseline patient and lesion characteristics
Baseline patient and lesion characteristics are listed in Table 1. The angiographic diameter stenosis of the lesions analysed was 64 ± 26% (visual estimation).

Virtual histology intravascular ultrasound analysis
VH-IVUS parameters are listed in Table 2. The mean analysed length was 49 ± 21 mm. The predominant plaque component over the whole analysis segment length was an Fi tissue, followed by an FF tissue, NC, and DC. The NC/DC ratio was a median of 1.79 (IQR 1.24–2.68).

Virtual histology intravascular ultrasound and lipid profile in men
We performed univariate analysis to determine the relationships between VH-IVUS and lipid profile parameters (Table 3). LDL-C showed a positive association with %Fi and mean Fi CSA. The TC/HDL ratio was also related to %Fi and mean FF CSA. There was a negative association between HDL values and %Fi and mean Fi CSA. Triglycerides had a positive relationship with %FF and mean FF CSA. The NC/DC ratio was consistently related to TC/HDL ratio, LDL-C levels, and HDL-C levels. There were no other significant correlations between VH-IVUS parameters and lipid profile.

Patients with abnormal HDL-C (<50 mg/dL) levels had larger mean Fi CSA (2.47 ± 1.43 vs. 2.0 ± 1.21 mm², P = 0.005) and NC (0.46 ± 0.38 vs. 0.37 ± 0.37 mm², P = 0.03), but not mean FF or DC CSA (Figure 1). The same pattern was found for measures of per cent plaque component volume. There were no
significant differences in plaque components for patients with normal vs. abnormal TC or LDL-C levels. However, patients with abnormal TG levels had plaques with a higher FF mean CSA (0.93 ± 0.70 vs. 0.73 ± 0.54 mm², P = 0.008), higher %FF plaque volume (11.4 ± 6.4 vs. 9.7 ± 4.8%, P = 0.01), and less %DC plaque volume (5.2 ± 3.8 vs. 5.9 ± 3.8%, P = 0.02). There were no significant differences for any of VH-IVUS parameters or the NC/DC ratio when patients were separated according to statin use.

The NC/DC ratio was significantly higher for patients with abnormal vs. normal LDL-C levels, TG levels, TC/HDL levels, but not for HDL-C levels (Table 4). Patients with TC/HDL levels in the third tertile had a significantly higher NC/DC ratio [median 2.18 (IQR 1.40–3.25)] when compared with patients in the first [median 1.65 (IQR 1.05–2.44)] and second tertiles [median 1.66 (IQR 1.18–2.33)] (P = 0.004). The NC/DC medians were progressively higher according to LDL-C tertiles (P = 0.01) (Figure 2).

**Virtual histology intravascular ultrasound vs. risk factors pathologically linked to sudden coronary death in men**

We separated male patients according to previously described clinical correlates of high-risk sudden-coronary-death plaque morphologies. Patients with a TC/HDL > 5 and/or TC > 210 mg/dL had a higher %FI content and a higher NC/DC ratio when compared with patients without those risk factors (Table 5). The NC/DC was significantly higher for smokers [median 1.98 (IQR 1.35–3.18)] vs. non-smokers [median 1.70 (IQR 1.23–2.53), P = 0.006]. Other VH parameters were not significantly influenced by smoking. Therefore, the only VH-IVUS parameter consistently elevated in male patients with a risk profile for SCD was the NC/DC ratio.

We used multiple logistic regression to predict a VH-IVUS risk profile associated with clinical correlates of SCD (TC/HDL > 5 or cigarette use). All individual VH parameters, expressed as percentage of plaque volume, and the NC/DC ratio were tested as continuous independent variables. Only the NC/DC ratio was identified as an independent predictor (P = 0.03), whereas %FI (P = 0.08), %DC (P = 0.16), %NC (P = 0.39), and %FF (P = 0.39) were not significant predictors in this model.

A second analysis was then performed to identify an NC/DC ratio cutoff that best separated patients with vs. without correlates of a pathology-related risk profile for SCD (TC/HDL ratio > 5 and/or current smoking). An NC/DC > 3 had a sensitivity of 33% and a specificity of 86% (odds ratio 3.0, 95% CI 1.7–4.9, P < 0.0001) (Table 6). Finally, receiver-operator curves were plotted to demonstrate that an NC/DC ratio presented a better performance than isolated measures of %NC or %DC to identify these patients (Figure 3).

**Discussion**

Sudden cardiac death is a major cause of mortality in the USA, accounting for more than 300 000 deaths annually. It is the most common and often the first manifestation of CAD, and is responsible for 50% of the cardiovascular disease mortality in the USA and other developed countries. The epidemiology of sudden cardiac death in adults parallels that of CAD: (i) up to 80% of individuals who suffer sudden cardiac death have CAD, (ii) the peak occurrence of sudden cardiac death occurs between 45 and 75 years of age, and (iii) sudden cardiac death has a much higher incidence in men than in women, reflecting sex differences in the incidence of CAD as well (~75% of sudden cardiac deaths occur in men).21

Age, hypertension, left ventricular hypertrophy, intraventricular conduction block, elevated serum cholesterol, glucose intolerance, decreased vital capacity, smoking, relative weight, and heart rate identify individuals at risk for sudden cardiac death, regardless of
the underlying cause—coronary disease, non-ischaemic myocardial diseases, structural heart disease, or primary electrophysiology disturbances. However, pathological findings in sudden death caused specifically by acute coronary events show that serum cholesterol levels, smoking, and menopausal status play a major role. Burke et al. demonstrated that rupture of high-risk coronary plaque morphologies and acute coronary thrombosis in men who died suddenly were related to abnormal cholesterol levels and smoking, but not to other risk factors such as glycaemic control, hypertension, or age. The authors found that the number of vulnerable plaques had a positive association to the TC/HDL ratio. Furthermore, smoking was an independent predictor in 59 patients who died of acute coronary thrombosis (odds ratio 3.6, \(P = 0.0004\)). Thus, we based our analysis on these previously reported results. Our major findings were that the NC/DC ratio was the parameter consistently related to a high-risk lipid profile, and that an NC/DC > 3 identifies patients with a risk profile for SCD (smoking and/or TC/HDL > 5) with high specificity (86%).

Table 3 Univariate analysis showing the relationship of serum lipids with virtual histology intravascular ultrasound parameters in male patients

<table>
<thead>
<tr>
<th>VH-IVUS parameters</th>
<th>TC R-value</th>
<th>P-value</th>
<th>HDL-C R-value</th>
<th>P-value</th>
<th>LDL-C R-value</th>
<th>P-value</th>
<th>TC/HDL ratio R-value</th>
<th>P-value</th>
<th>Triglycerides R-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean FI CSA, mm²</td>
<td>0.06</td>
<td>0.29</td>
<td>-0.14</td>
<td>0.008</td>
<td>0.12</td>
<td>0.03</td>
<td>0.17</td>
<td>0.001</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>Mean FF CSA, mm²</td>
<td>0.05</td>
<td>0.37</td>
<td>0.04</td>
<td>0.48</td>
<td>0.07</td>
<td>0.21</td>
<td>0.13</td>
<td>0.01</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean NC CSA, mm²</td>
<td>0.05</td>
<td>0.36</td>
<td>0.11</td>
<td>0.05</td>
<td>0.01</td>
<td>0.84</td>
<td>0.09</td>
<td>0.11</td>
<td>0.05</td>
<td>0.40</td>
</tr>
<tr>
<td>Mean DC CSA, mm²</td>
<td>0.09</td>
<td>0.12</td>
<td>0.03</td>
<td>0.96</td>
<td>-0.08</td>
<td>0.14</td>
<td>-0.02</td>
<td>0.63</td>
<td>0.05</td>
<td>0.39</td>
</tr>
<tr>
<td>% FI volume</td>
<td>0.08</td>
<td>0.15</td>
<td>-0.15</td>
<td>0.005</td>
<td>0.13</td>
<td>0.01</td>
<td>0.19</td>
<td>0.003</td>
<td>0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>% FF volume</td>
<td>0.02</td>
<td>0.73</td>
<td>-0.06</td>
<td>0.24</td>
<td>-0.002</td>
<td>0.97</td>
<td>0.03</td>
<td>0.51</td>
<td>0.17</td>
<td>0.01</td>
</tr>
<tr>
<td>% NC volume</td>
<td>0.05</td>
<td>0.32</td>
<td>0.11</td>
<td>0.05</td>
<td>0.01</td>
<td>0.86</td>
<td>0.06</td>
<td>0.26</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td>% Calcified volume</td>
<td>0.11</td>
<td>0.05</td>
<td>-0.01</td>
<td>0.82</td>
<td>-0.11</td>
<td>0.06</td>
<td>-0.06</td>
<td>0.21</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>NC/DC ratio</td>
<td>0.08</td>
<td>0.10</td>
<td>-0.11</td>
<td>0.03</td>
<td>0.17</td>
<td>0.02</td>
<td>0.18</td>
<td>0.0008</td>
<td>0.06</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*Spearman rank correlation was performed. All other associations were tested using simple regression analysis.

Table 4 Virtual histology intravascular ultrasound necrotic core/dense calcium ratio for male patients according to abnormal vs. normal lipid profile parameters

<table>
<thead>
<tr>
<th>Lipid profile parameters</th>
<th>NC/DC ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC/HDL ratio &gt; 5</td>
<td>2.18</td>
<td>1.39–3.61</td>
</tr>
<tr>
<td>TC/HDL ratio ≤ 5</td>
<td>1.69</td>
<td>1.17–2.44</td>
</tr>
<tr>
<td>LDL-C, mg/dL ≥ 100</td>
<td>1.87</td>
<td>1.26–3.12</td>
</tr>
<tr>
<td>LDL-C, mg/dL &lt; 100</td>
<td>1.58</td>
<td>1.16–2.32</td>
</tr>
<tr>
<td>HDL-C, mg/dL ≥ 50</td>
<td>2.00</td>
<td>1.28–1.09</td>
</tr>
<tr>
<td>HDL-C, mg/dL &lt; 50</td>
<td>1.89</td>
<td>1.09–2.48</td>
</tr>
<tr>
<td>Triglycerides, mg/dL ≥ 150</td>
<td>1.97</td>
<td>1.44–3.40</td>
</tr>
<tr>
<td>Triglycerides, mg/dL &lt; 150</td>
<td>1.67</td>
<td>1.17–2.60</td>
</tr>
</tbody>
</table>

Cigarette smoking and high-cholesterol levels: key risk factors in the search for the ‘vulnerable patient’?

In the Framingham study, the annual incidence of sudden cardiac deaths increased from 13 per 1000 in non-smokers to almost 2.5 times that value for people who smoked more than 20 cigarettes per day, emerging smoking as one of the major cardiovascular risk factors with the largest impact on unexpected cardiac mortality. It is also an important risk factor when associated with other major coronary risk factors. In a large pooled analysis of more than 350 000 men from the Chicago cohorts study, Stamler et al. reported an independent relationship of baseline cigarette use, serum cholesterol, and blood pressure to risk of death from CAD. Moreover, the combination of these three risk factors resulted in an increase of 69% in risk of CHD death, with a reduction in longevity.
Calcium, necrotic core, and plaque instability

Lipid-lowering therapy reduces the incidence of acute coronary events and death. High LDL-C serum levels increase the availability of LDL-C to the vessel wall, predisposing macrophages to uptake cholesterol from apolipoprotein B-containing lipoproteins, forming the lipid-rich NC. Conversely, an increase in serum HDL-C accelerates reverse cholesterol transport, decreasing the NC size, and potentially stabilizing plaque. In our analysis, we found less NC in diseased segments of patients with normal versus abnormal serum HDL-C, but there was no significant correlation of HDL-C (or other lipid parameters) levels with NC size. On the other hand, the ratio of NC to DC was consistently affected by TC/HDL ratio, triglycerides, LDL-C, and HDL-C. Smaller NCs and more calcified tissue (i.e., a reduced ratio of NC to DC) could be a consequence of ‘healing’ or ‘stabilizing’ of previous vulnerable plaques—exactly the pattern found for patients with a benign lipid profile in our study.

There is a lack of studies looking at the dynamic relationship of calcium and NC in terms of plaque instability, but both plaque components have been extensively studied in vivo and ex vivo as individual parameters. Coronary calcification correlates with plaque burden, but its effect on plaque instability is less evident. Beckman et al. demonstrated that the maximal arc of calcium decreased progressively from patients with stable angina (91 ± 10°) to those with unstable angina (59 ± 8°) and to those with MI (49 ± 11°; P = 0.014). Ehara et al., in a study of preinterventional IVUS in 178 patients, reported that the average number of calcium deposits within an arc of <90° decreased progressively from patients with acute MI to unstable angina to stable angina (P < 0.0005). Calcium deposits were also significantly longer in stable angina patients. Fuji et al. analysed 101 ruptured plaques by IVUS and showed that ruptured plaques also had quantitatively less calcium, but a larger number of small calcium deposits when compared with non-ruptured plaques. Conversely, pathological reports have shown that the percentage of NC found in different lesion morphologies has a positive relationship with plaque vulnerability: the highest lesion-site NC is found in ruptured plaques (34 ± 17%), followed by thin-cap fibroatheroma (24 ± 17%), plaque erosion (14 ± 14%), and stable plaques (12 ± 25%). Huang et al. studied the impact of calcification and NC on biomechanical stability of stable and ruptured plaques. The authors found that large amounts of calcium decreased stresses on the Fi plaque only to a modest degree, suggesting that calcification does not decrease plaque stability; in fact, removal of larger amounts of calcification resulted in a less stable atheroma. Conversely, maximum stress was positively correlated with the percentage of lipid (P = 0.02). Our finding in vivo that an NC/DC ratio may play a major role in terms of plaque instability is consistent with this concept.

Study limitations

This study has several limitations. First, the current VH-IVUS tree is not able to differentiate intraluminal thrombus from other plaque components. Second, this is a cross-sectional study and lacks clinical follow-up. Third, this study focuses only on VH-IVUS.
parameters, and biomarkers such as hs-C-reactive protein were not tested. Fourth, we analysed an ‘arbitrary’ segment of one culprit coronary artery, rather than the proximal segments of all three major epicardial arteries.

Conclusions

The ratio of NC to calcification detected by VH-IVUS in diseased coronary segments is related to known risk factors for SCD in men, and thereby may be associated with a worse prognosis.

Conflict of interest: G.S.M. is a member of speakers’ bureau for Boston Scientific Corporation, has ownership interest, and is a consultant for Volcano Corporation; G.W.S. is a consultant for Boston Scientific Corporation and Volcano Corporation; M.B.L. has ownership interest for Volcano Corporation and is a consultant for Boston Scientific Corporation.

Funding

Volcano Corporation (Rancho Cordova, CA, USA) has sponsored this registry.

Appendix: Volcano VH-IVUS Registry participating centres

USA: Mid America Heart, Kansas City, MO; Mayo Clinic, MN; Mt Sinai, Miami, FL; Columbia Medical Center, New York; Pinnacle Health, Harrisburg, PA; UC Davis, CA; Mt Clemens, MI; Arizona Heart, Phoenix, AZ; Cleveland Medical Foundation, Cleveland, OH; Winchester Medical, VA; Forsyth Medical Center, NC; St Louis University, MO; St Francis, IN; Nebraska Heart, NE.

Europe: Klinikum Innenstadt, Munich; Herz- und Neurozentrum, Kreuzlingen; Haukeland University Hospital, Bergen; OLVZ, Aalst; University Hospital, Essen; Ichilov, Tel Aviv; Hospital Santa Marta, Lisbon; AKH, Vienna; Cliniche Gavazzeni, Bergamo; University Hospital Leiden, Leiden; Mexicordion Hospital, Vigo; Valdecilla, Santander; Clinico San Carlos, Madrid; Jagellonian University Hospital, Krakow.
Virtual histology and sudden coronary death

References