Do systemic risk factors impact invasive findings from virtual histology? Insights from the international virtual histology registry

Sebastian Philipp1*, Dirk Böse1†, William Wijns2, Steven P. Marso3, Robert S. Schwartz4, Andreas König5, Amir Lerman6, Hector M. Garcia-Garcia7, Patrick W. Serruys7, and Raimund Erbel1

1Department of Cardiology, Westgerman Heart Center Essen, University Duisburg-Essen, Hufelandstr. 55, D-45122 Essen, Germany; 2Department of Cardiology, Cardiovascular Centre, Aalst, Belgium; 3Mid America Heart Institute, Saint Luke’s Hospital, Kansas City, MO, USA; 4Minneapolis Heart Institute and Foundation, Minneapolis, MN, USA; 5Division of Cardiology, Department of Medicine, Medizinische Klinik und Poliklinik-Innenstadt, Munich, Germany; 6Division of Cardiovascular Diseases, Mayo Clinic College of Medicine, Rochester, MN, USA; and 7Erasmus Medical Center, Thoraxcenter, Rotterdam, The Netherlands

Received 13 March 2009; revised 16 July 2009; accepted 20 August 2009; online publish-ahead-of-print 23 October 2009

Aims
Cardiovascular risk factors such as elevated serum lipid levels are important in the development of coronary atherosclerosis. Radiofrequency (RF) analysis of intravascular ultrasound [IVUS, Virtual histology™ (VH)] offers a unique tool to study the composition of coronary atherosclerotic plaque in vivo. We used data from the multicentre VH registry to assess the association between cardiovascular risk factors and coronary plaque volume and composition.

Methods and results
Between August 2004 and July 2006, 990 patients in 42 centres were enrolled in a prospective, multicentre, non-randomized global VH registry. Coronary artery imaging was performed by conventional IVUS and RF-IVUS. The four RF-IVUS plaque components [dense calcium (DC), necrotic core (NC), fibrous (F) tissue, and fibro fatty (FF)] were analysed in every recorded frame. The results were expressed as mean cross-sectional areas, absolute volume, and percentage of total plaque volume. Risk factor assessment included evaluation of family history of previous myocardial infarction (MI), past or current smoking, diabetes mellitus, hypertension, and the laboratory measurements. Patients with diabetes had an increased relative proportion of NC (6.47 ± 0.28 vs. 5.86 ± 0.14%, P = 0.037) and DC (4.58 ± 0.27 vs. 3.90 ± 0.14%, P = 0.017), and patients with hypertension had an increased relative proportion of FF, DC (4.35 ± 0.16 vs. 3.57 ± 0.17%, P = 0.02) and NC (6.24 ± 0.17 vs. 5.60 ± 0.19%, P = 0.01). Compared with patients with LDL-C <100 mg/dL, patients with LDL-C >160 mg/dL had higher plaque volume (342.1 ± 26.2 vs. 318.6 ± 10.7 mm³). Linear regression analysis showed a correlation between the level of HDL-C and F (r = −0.149, P < 0.01), FF (r = −0.106, P < 0.01), and NC (r = −0.90, P < 0.05). The level of LDL correlated with F (r = 0.110, P < 0.01). Patients with prior MI have an increased percentage of F (30.03 ± 0.59 vs. 28.20 ± 0.37%, P = 0.009). Smoking had no relevant effect on plaque composition. Treatment with acetylsalicylic acid and statins reduced FF with altering plaque volume.

Conclusion
Radiofrequency-IVUS detects marked differences in coronary plaque composition related to the risk factor profile with particular focus on lipid levels. Greater amounts of NC were associated with diabetes, hypertension, MI, and low HDL-C. The effects of treatment of changes related to plaque composition are underway.

Keywords
Intravascular ultrasound • Radiofrequency • Virtual histology • Plaque composition • Risk factors
Introduction

Most cardiovascular events are clinical manifestations of underlying atherosclerotic disease, and cardiovascular risk factors are associated with coronary artery disease (CAD). Atherosclerotic plaque is much larger and more heterogeneous than suspected from the coronary angiographic findings.1 The discrepancy can be partially explained by remodelling of coronary arteries.2

The amount of plaque derived from grey-scale IVUS data has been shown to correlate with risk factors, the effect of systemic therapy, and prognosis.3–5 It has been demonstrated previously that mean percentage of plaque burden and plaque area correlates with known risk factors (diabetes, hypertension, hyperlipidaemia, and smoking) for CAD and ischaemia demonstrating a more advanced stage of CAD in these patients.6–8

Vulnerable plaques are high-risk atherosclerotic lesions and complications of these plaques (plaque rupture, luminal and mural thrombosis, intraplaque haemorrhage, rapid progression in stenosis severity, spasm, etc.) lead to acute coronary syndrome (ACS).9–14 Unstable, non-calcified lipid-rich plaques are believed to play a major role in these events and the ability to quantify the amount necrotic lipid core in lesions maybe a potential measure for further risk stratification.15,16 Traditional coronary imaging modalities, including angiography and intravascular ultrasound (IVUS), provide limited information on the vulnerability of atherosclerotic plaques.17–19 Virtual histologyTM (VH), a novel IVUS-derived technology, is based on the analysis of radiofrequency signals (RF-IVUS) to identify and quantify various plaque components; it has an 93–97% ex vivo and 87–92% in vivo accuracy for characterization of four basic plaque components: fibrous (F) tissue, fibro-fatty (FF) plaque, dense calcium (DC), and a lipid-rich necrotic core (NC).20–27

The purpose of this study was to assess the impact of cardiovascular risk factors, in particular serum lipid levels, not only on overall coronary plaque volume but particularly on coronary plaque composition. We also investigated the impact of systemic medication on plaque composition.

Methods

Population: Volcano registry

Between August 2004 and July 2006, 990 patients from 42 centres were enrolled in a prospective, multicentre, global registry (VH registry). The Ethics Committee at each of the participating institutions approved the protocol, and written informed consent was obtained from these patients. Patients were eligible to participate in the registry if they were at least 18 years old and if there were no contraindications for IVUS. The indication for the catheterization was set by the investigator either based on a positive functional test or symptoms of angina.

Risk factors

Risk factor assessment included evaluation of family history of premature myocardial infarction (MI), past or current smoking, diabetes mellitus, hypertension, and the laboratory measurements described below. A lipid disorder was defined as a total cholesterol (TC) level of ≥200 mg/dL, LDL-C ≥100 mg/dL, HDL-C <50 mg/dL, triglycerides (TG) ≥150 mg/dL, or lipid lowering 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 medication. Patients with diabetes mellitus had a confirmed diagnosis or were using antidiabetic medications (insulin or oral hypoglycaemic) at study entry. There was no further definition for family history.

Assessment of coronary plaque composition with intravascular ultrasound-virtual histology

The vessel chosen for the IVUS investigation was identified by the investigator and the definition for this decision was based on the intention of the catheterization. A commercially available phased-array, 20 MHz-IVUS catheter (Eagle Eye® Gold, Volcano Corporation, Rancho Cordova, CA, USA), was placed distal into the target vessel, and a motorized pullback through the vessel was performed at 0.5 mm/s up to the ostium of the guiding catheter. Geometrical IVUS measurements of external elastic membrane, plaque and media and lumen cross-sectional areas (CSA), and plaque burden (plaque and media divided by external elastic membrane) were performed for every recorded frame; volumes were calculated using Simpson’s rule. Intravascular ultrasound RF data were acquired with ECG gating at the peak of the R-wave; accordingly, the frame rate depended on the heart rate. The RF data of all patients had been archived on DVD for off-line analysis. The off-line analysis was performed using the pcVH 2.2 software (Volcano Corporation). The initial border editing for most of the cases was performed in Volcano’s internal corelab in Cleveland by a dedicated engineer. Afterwards, all cases were reviewed and finally approved in the two corelabs: data acquired in the USA and in Japan were approved in the Cardiovascular Research Foundation (CRF), New York. Data acquired in Europe was approved in Cardialysis, Rotterdam. Some cases of these two groups (USA and Japan, and Europe) were entirely analysed in either CRF or Cardialysis.

Spectral analysis of radiofrequency ultrasound backscatter intravascular ultrasound signal

For each IVUS frame, plaque characterization was obtained within these borders. The software differentiated between tissue types and assigned colour codes to them: (i) F tissue (labelled green), (ii) FF tissue (labelled greenish-yellow), (iii) NC (labelled red), and (iv) DC (labelled white). In order to provide comparable volumetric data, we present for each individual plaque component not only the absolute volume, but also the proportion of the total plaque volume (%) and the volume normalized for 10 mm of segment length. The four VH-IVUS plaque components were measured in every recorded frame and for the imaged segment expressed as mean CSA, absolute volume, and percentage of total plaque volume.

Statistical analysis

Statistical analysis was performed with SPSS v. 15.0 (SPSS Inc., Chicago, IL, USA; SAS Institute, Cary, NC, USA). Pearson’s correlation coefficients were used to analyse univariate correlations between independent variables. Categorical data were expressed as numbers or frequencies and compared using χ² statistics. Continuous data were reported as mean ± SD or as median with inter-quartile range. Unpaired Student’s t-test or analysis of variance was used to compare two or more sets of data with normal distribution. If normality failed, the Mann–Whitney U statistic or Kruskal–Wallis test was performed. A P-value <0.05 was considered statistically significant.
Results

Study population and baseline characteristics

In a prospective, multicentre, non-randomized, global VH-IVUS registry, 990 patients in 42 centres were involved in this analysis. Patient demographics, clinical presentation, and procedure characteristics are presented in Table 1. 24.5% of the patients were females. Almost two-thirds of the patients had hypertension, about one-fourth were diabetics (6.2% insulin-dependent), and 25.8% current smokers. A positive family history of CAD was found in 43.1%, and 65.2% had dyslipidaemia. Most of the patients investigated had stable angina (43.6%) or had no symptoms of CAD (15.6%). About 187 patients (18.0%) presented with an acute MI, and 215 patients (21.7%) had unstable angina according to the Braunwald classification. Of the lesions investigated, 81.2% (804) were de novo and 10.7% (105) were restenotic (Figure 1). Of the vessels investigated, 36.2% (358) were pre-intervention, 43.1% (428) were diagnostic, 6.4% (63) were post-balloon-dilatation (of a different vessel), 12.9% (128) were post-stent (of a different vessel), and 1.3% (13) were other. Laboratory parameters included a mean LDL-C of 106 ± 1.4 mg/dL and a median HDL of 47 ± 0.6 mg/dL.

Geometrical and tissue composition vessel characteristics

Intravascular ultrasound characteristics are listed in Table 2. In the whole segment length, the predominant plaque component for the group of 990 patients was F tissue, followed by FF plaque, NC, and DC. The NC/DC ratio was a median of 1.74 (inter-quartile range, 1.18–2.58). Levels of biomarkers such as creatinine kinase and C-reactive protein showed no association with plaque burden or plaque composition at all.

Age and gender

Age was not associated with plaque volume. But there was a relevant change in the plaque composition. Elderly patients had an increased percentage of DC (r = 0.233, P < 0.0001) and of NC (r = 0.171, P < 0.0001). Furthermore, males had significantly higher plaque volume (349.2 ± 7.7 vs. 287.5 ± 11.8 mm³, P < 0.001) than females with a significant higher percentage of F and FF but a decrease in DC. There was no difference in NC (Figure 2).

Cardiovascular risk factors analyses

Dyslipidaemia and smoking

Linear regression analysis showed a negative correlation between the level of HDL-C and F and FF plaque and a positive correlation between the level of LDL and F and FF plaque (Table 3). Compared with patients with LDL-C <100 mg/dL, patients with LDL-C >160 mg/dL had higher plaque volume (342.1 ± 26.2 vs. 318.6 ± 10.7 mm³, P = 0.01). Smoking was not associated with a specific plaque composition in this study population.

Diabetes and hypertension

Compared with patients without diabetes, patients with diabetes (n = 243) had no differences in plaque volume, F-, FF-, DC-, or...
Impact of risk factors on plaque composition

Table 2 Geometrical and compositional vessel characteristics

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std</th>
<th>Median</th>
<th>Inter-quartile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel volume (mm³)</td>
<td>751</td>
<td>415</td>
<td>671</td>
<td>466–946</td>
</tr>
<tr>
<td>Lumen volume (mm³)</td>
<td>417</td>
<td>240</td>
<td>368</td>
<td>258–521</td>
</tr>
<tr>
<td>Plaque volume (mm³)</td>
<td>334</td>
<td>206</td>
<td>286</td>
<td>195–428</td>
</tr>
<tr>
<td>% DS (angio)</td>
<td>63</td>
<td>20</td>
<td>62</td>
<td>51–75</td>
</tr>
<tr>
<td>Average vessel CSA (mm²)</td>
<td>15.56</td>
<td>4.8</td>
<td>15.03</td>
<td>12.30–18.06</td>
</tr>
<tr>
<td>Average lumen CSA (mm²)</td>
<td>8.72</td>
<td>2.96</td>
<td>8.17</td>
<td>6.54–10.26</td>
</tr>
<tr>
<td>Average plaque area CSA (mm²)</td>
<td>6.84</td>
<td>2.75</td>
<td>6.45</td>
<td>4.98–8.22</td>
</tr>
<tr>
<td>Average per cent stenosis</td>
<td>44</td>
<td>9</td>
<td>44</td>
<td>37–49</td>
</tr>
<tr>
<td>Average fibrous CSA (mm²)</td>
<td>2.15</td>
<td>1.43</td>
<td>1.85</td>
<td>1.17–2.83</td>
</tr>
<tr>
<td>Average fibro-fatty CSA (mm²)</td>
<td>0.70</td>
<td>0.59</td>
<td>0.55</td>
<td>0.30–0.93</td>
</tr>
<tr>
<td>Average necrotic core CSA (mm²)</td>
<td>0.45</td>
<td>0.42</td>
<td>0.34</td>
<td>0.16–0.62</td>
</tr>
<tr>
<td>Average calcified CSA (mm²)</td>
<td>0.31</td>
<td>0.38</td>
<td>0.20</td>
<td>0.07–0.41</td>
</tr>
</tbody>
</table>

Figure 2 Gender differences in plaque composition. The arteries of males were significantly less calcified but had a higher percentage of fibrous and fibro-fatty tissue. The necrotic core was unchanged. *P < 0.05 vs. the corresponding value in males.

NC-volume. But there was a significantly higher percentage of DC (4.58 ± 0.27 vs. 3.90 ± 0.14%, P = 0.017) and that of NC (6.47 ± 0.28 vs. 5.86 ± 0.14%, P = 0.037) in patients with diabetes. Focusing on the patients with insulin-dependent diabetes (n = 61), there was a further increase in the percentage of DC (5.01 ± 0.60%) and that of NC (7.07 ± 0.061%) (Figure 3). Patients with hypertension (n = 629) had a significant increase in FF, DC, and NC compared with patients without hypertension (Figure 4).

Family predisposition, previous coronary artery bypass grafting, and previous myocardial infarction

Patients with a positive family history of CAD had no difference in plaque volume or plaque composition. In patients with previous MI, there was no difference in plaque volume or in the percentage of FF, DC, and NC. Interestingly, there was a mild, but significant increase in F tissue percentage (30.03 ± 0.59 vs. 28.20 ± 0.37%, P = 0.009). The arteries of patients with prior coronary artery bypass grafting (CABG) were significantly more calcified and had more NC than the arteries of patients without previous CABG (Figure 5).

Influence of treatment with acetylsalicylic acid and statins

Plaque volume, DC, or NC were not different between groups treated with and without acetylsalicylic acid (ASA) and/or statins. There was a significant reduction in FF and the combination of ASA and statins (Figure 6).

Discussion

In the present investigation, the ‘classic’ five cardiovascular risk factors were investigated and correlated with the plaque composition of the respective patients. We also investigated the influence of age, gender, and past medical history such as previous MI and previous CABG on plaque burden and plaque composition. This analysis involved 990 patients from over 40 international centres. The differences seen were rather small but of interest. Radiofrequency-IVUS detected marked differences in coronary plaque composition related to the risk factor profile with particular focus on lipid levels. Greater amounts of NC were associated with hypertension, MI, low HDL-C, diabetes, and patients with previous CABG.

In previous RF-IVUS studies, the focus was on patients with ACS. Rodriguez-Granillo et al.24 showed that non-culprit lesions (mild to moderate angiographic stenosis) of patients with ACS had a larger percentage of NC (12.26 ± 7.0 vs. 7.40 ± 5.5%, P = 0.006) and less fibrotic tissue (63.96 ± 9.1 vs. 70.97 ± 9.3%, P = 0.007) compared with secondary lesions of patients with stable angina. Surmely et al.28 reported the opposite findings, i.e. more fibrous tissue (66.0 ± 10.7 vs. 61.4 ± 8.9%, P = 0.03) and less NC (6.8 ± 6.0 vs. 11.0 ± 8.3%, P = 0.02) at the minimal lumen area of culprit ACS vs. stable angina lesions, also contradicting previous histopathologic reports. Histopathologic studies have shown that progressively greater lesion instability is related to the percentage of NC found in different lesion morphologies. The highest lesion-site percentage of NC is found in ruptured vulnerable plaques (34 ± 17%), followed by thin-cap fibroatheromas (24 ± 17%), plaque erosion (14 ± 14%), and stable plaques (12 ± 25%).29 Previous epidemiologic studies and post-mortem studies have taught us that diabetes, hypertension, previous cardiac history, and multivessel disease are some of the most common risk factors for a subsequent ischaemic cardiac event. In addition, post-mortem data have shown that the NC together with...
calcium are the most dangerous plaque components with regard to sudden plaque rupture and subsequent ischaemic coronary death.9 It is known that diabetics have an increased risk for cardiovascular events. Frutkin et al. analysed coronary artery lesions of diabetics and a matched control group by VH-IVUS. Both groups had similar plaque geometry and remodelling indices but diabetic coronary lesions had significantly more NC and calcium content.30 We were able to confirm these findings in a much larger population with the addition that patients with insulin-dependent diabetes had even more NC and DC. Missel et al. assessed the relationship of lipid profile to VH-IVUS. Whole vessel IVUS analysis was used to measure external elastic membrane, lumen, plaque and media CSA and volumes, and calculated mean plaque burden in a series of 625 patients. In that study, TC, LDL-C, or TG were not related to any of these IVUS parameters. Multiple regressions showed that HDL-C was the only independent predictor of mean plaque burden, which we can confirm, although we also showed an effect of LDL-C on plaque volume and DC. Thus, the limited benefit of statins on plaque burden and composition can be explained.

The Second Northwick Park Heart Study (NPHS-II) and the Reykjavik Cohort Study showed that a positive family history for CAD increases the risk of MI by 60%.31,32 Nevertheless, in our investigation, we saw no difference in plaque burden and composition in patients with a genetic risk for CAD. Further, patients with previous MI had a lower content of F tissue percentage without differences in FF, DC, NC, or plaque burden. But patients with a severe CAD and previous CABG had significantly more plaques with DC and NC. Calcification and NC seem to be a strong predictor for a more advanced stage of CAD, particularly if insulin dependent diabetes mellitus and low HDL-C are taken into account.

**Limitations**

Limitations of VH-IVUS include the separation of NC from DC. In the initial study performed by Nair et al.,22 NC and DC were summarized into calcified necrosis. The degree of calcification in large cores is quite variable with small calcifications of macrophages up to irregular large blocks of calcium. Due to involvement of adjacent fibrous structures, the differentiation to NC is rather difficult. Thrombus may be misclassified as fibrous plaque

---

**Table 3** Correlation between dyslipidaemia and plaque composition

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average plaque + media (CSA)</td>
<td>0.03</td>
<td>0.08*</td>
<td>−0.13**</td>
</tr>
<tr>
<td>Plaque volume</td>
<td>0.01</td>
<td>0.05</td>
<td>−0.01*</td>
</tr>
<tr>
<td>Average fibrous (CSA)</td>
<td>0.06 (P = 0.78)</td>
<td>0.11**</td>
<td>−0.15**</td>
</tr>
<tr>
<td>Average fibro-fatty (CSA)</td>
<td>0.03</td>
<td>0.07 (P = 0.63)</td>
<td>−0.11**</td>
</tr>
<tr>
<td>Average calcified (CSA)</td>
<td>−0.07*</td>
<td>−0.08*</td>
<td>−0.03</td>
</tr>
<tr>
<td>Average necrotic core (CSA)</td>
<td>−0.02</td>
<td>0.01</td>
<td>−0.90*</td>
</tr>
</tbody>
</table>

This table demonstrates the correlation between plaque-components and volume and lipids. Patients with high cholesterol and LDL have less calcified lesions but have more fibrous tissue. Patients with elevated HDL have less plaque, F, FF, and NC. CSA, cross-sectional area; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

*P < 0.05, **P < 0.01 vs. the corresponding value in patients with normal cholesterol, LDL, and HDL.

---

**Figure 3** Necrotic core and calcium are increased in diabetics. Dependent on the severity of the diabetes, the arteries were more calcified and necrotic. *P < 0.05 vs. the corresponding value in non-diabetic.

**Figure 4** Hypertension is associated with increased fibro-fatty, calcified, and necrotic plaque. Patients with hypertension tend to have more fibro-fatty tissue in their plaque. The arteries were more calcified and necrotic. *P < 0.05 vs. the corresponding value in normotensive patients.
to analyse plaque and lesions with the ‘gold standard’ of histopathology findings which are CABG. Women, but also patients with hypertension and previous insulin dependency, hypertension, and patients with previous CABG, had more calcified arteries. These findings primarily help to find new ways to look at disease progression and studies of pharmacological interventions.

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

Conflict of interest: none declared.

References

Impact of risk factors on plaque composition

Figure 5 Patients with prior coronary artery bypass grafting had more calcified and necrotic arteries. The arteries of patients with prior coronary artery bypass grafting were significantly more calcified and necrotic than the arteries of patients without previous coronary artery bypass grafting. *P < 0.05 versus the corresponding value in patients without coronary artery bypass grafting.

Figure 6 Only the combination of acetylsalicylic acid and statin reduced fibro-fatty. Only the combination of statin and aspirin reduced fibro-fatty. Only the combination of acetylsalicylic acid and statin was associated with less fibro-fatty plaque (*P < 0.05), and no difference was seen in the amount of necrotic core and calcium.

(fibro-fatty-dependent on age), proportionally increasing this plaque component at the expense of the others. Compared with the ‘gold standard’ of histopathology findings which are limited to autopsy series, we can use VH despite its limitations to analyse plaque and lesions in vivo which allow longitudinal trials looking at the effects of treatments and risk factors on plaque volume and composition over time.

The lack of longitudinal follow-up in this report is one limitation which should be addressed in upcoming investigations.

Summary
The data from the VH registry demonstrate that elevated NC is associated with gender, diabetes mellitus, and particular with insulin dependency, hypertension, and patients with previous CABG. Women, but also patients with hypertension and previous CABG, had more calcified arteries. These findings primarily help to find new ways to look at disease progression and studies of pharmacological interventions.


30. Frutkin AD, Mehta S, McCreary J, House H, Marso S. Patients with greater Framingham estimates of cardiac risk have increased coronary artery plaque burden without a change in relative plaque composition. *Am J Cardiol* 2007;100(Suppl. 8A):I32L.

