Review Article

Virtual Histology

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The term "virtual histology" is a concise term used to describe a technique complementary to intravascular ultrasound (IVUS), called 'radiofrequency backscatter spectral analysis'. IVUS Virtual Histology™ (IVUS-VH, Volcano Corporation, Rancho Cordova, CA, USA) is one of the three existing methods of analysis of IVUS backscattered tissue signal; the two other techniques, integrated backscatter analysis and wavelet analysis, have less theoretical and clinical documentation. IVUS-VH utilizes the IVUS signal to create color-coded maps that overlay traditional gray-scale IVUS images, aiming at distinguishing between areas of different histologic structure and thus offering information about the components of atheromatous plaques (Figure 1). The clinical usefulness of this information is evident and pertains to various aspects of coronary artery disease diagnosis and management.[1,2] For example, it has been shown that heavily calcified lesions are more prone to dissection when dilated during angioplasty[3] and that restenosis following a percutaneous intervention is more frequent in plaques with medial dissection.[4] Moreover, as will be discussed in more detail, it has become clear that angiographically mild lesions, when rich in necrotic and fatty material and with a thin fibrous cap, may give rise to acute coronary events.[5]

Virtual histology: the technique

An initial attempt to utilize backscattered IVUS tissue signal to acquire information regarding the histologic composition of vascular wall and atheromatous lesions involved quantification of the video intensity of imaged structures in the gray-scale images (videodensitometric analysis). However, this method is fraught with serious technical weaknesses, as the analyzed signal has already been processed in order to be transformed into a two-dimensional image. It is thus heavily distorted and significantly different from the initial acoustic signal.[6] In contrast, analysis of the unprocessed original backscattered signal (in both the amplitude and frequency domain) offers an inherently more accurate and reproducible technique of tissue characterization.[6-8]

Using data obtained from spectral analysis,[9-13] algorithms were developed in order to relate waveforms to specific histologic components of vascular lesions (Figure 2). The result of the analysis is then transformed into two-dimensional color-coded maps, with each color characterizing a different histologic area (hence the term "virtual histology"). Comparative studies showed a satisfactory correlation with true histology images.[14]

IVUS-VH discriminates between four distinct types of atherosclerotic plaque component: i) fibrous plaque, consisting

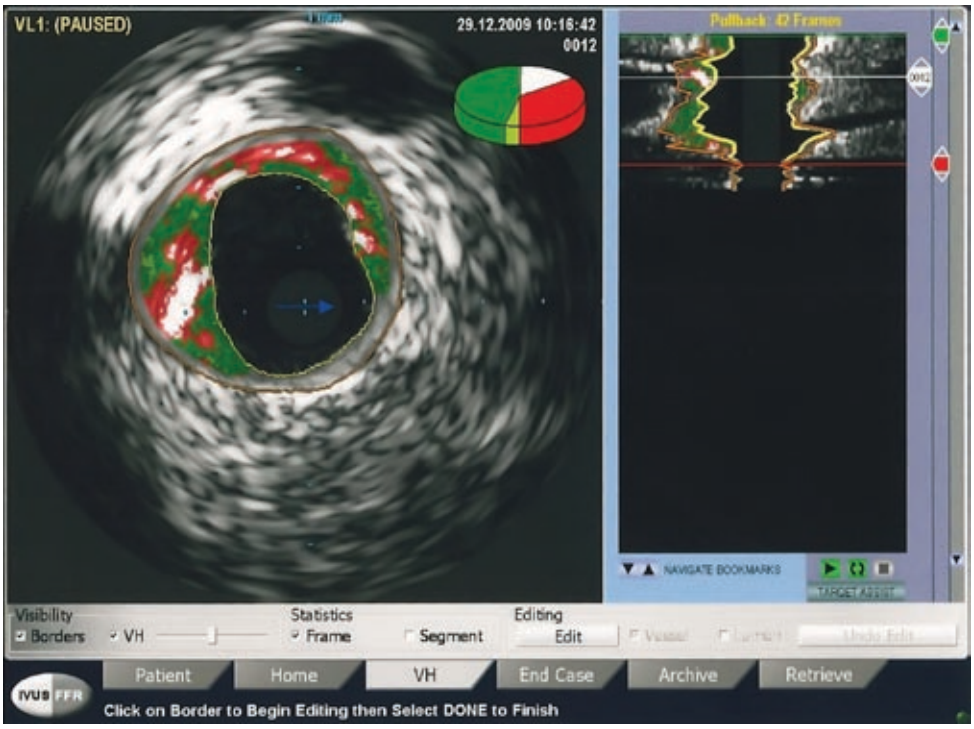


Figure 1. Screenshot of the IVUS working station during data processing for creating virtual histology images.

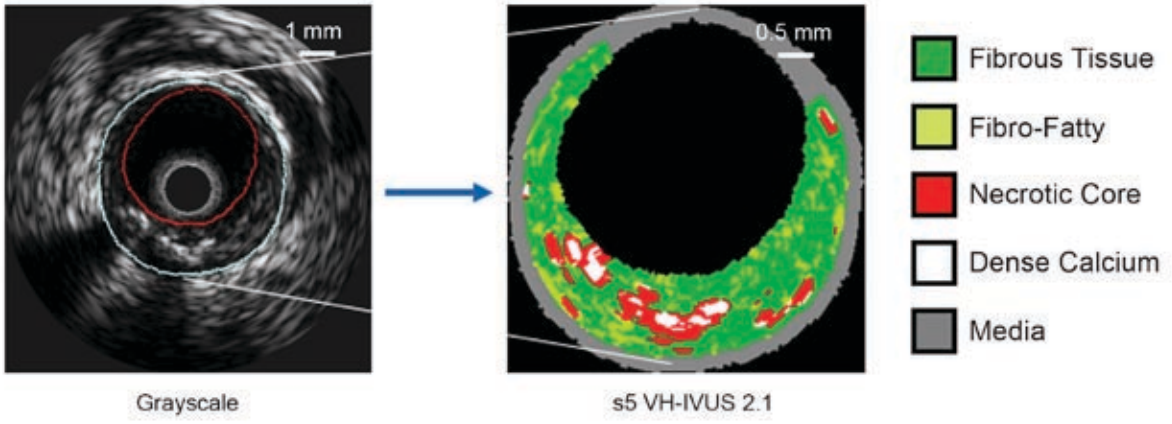


Figure 2. The four tissue types identified by IVUS-VH and their correspondence to histology. Courtesy of Volcano Co.

of dense collagen; ii) fibrofatty plaque, consisting of collagen interspersed with adipose tissue; iii) necrotic core, including clefts filled with cholesterol, foam cells and micro-calcific foci; and iv) calcified plaque.[14] Following spectral analysis, statistical processing, through a tree-like algorithm, attributes resulting waveforms to each one of the four histology types and creates a color image according to the following color-code: green (fibrous tissue), light green (fibrofatty tissue), red (necrotic core) and white (calcification) (Figure 3).

Comparison of IVUS-VH images to true histology sections from resected human coronary arteries showed a sensitivity and specificity of 80-92% in characterizing the four tissue types.[14] Rates of correct characterization by IVUS-VH, compared to coronary plaque sections obtained with directional coronary atherectomy, were 87% for fibrous tissue, 87% for fibrofatty tissue, 88% for necrotic core and 97% for calcified regions;[15] different rates have also been reported[16] (66.7% for fibrous tissue, 100% for fibro-

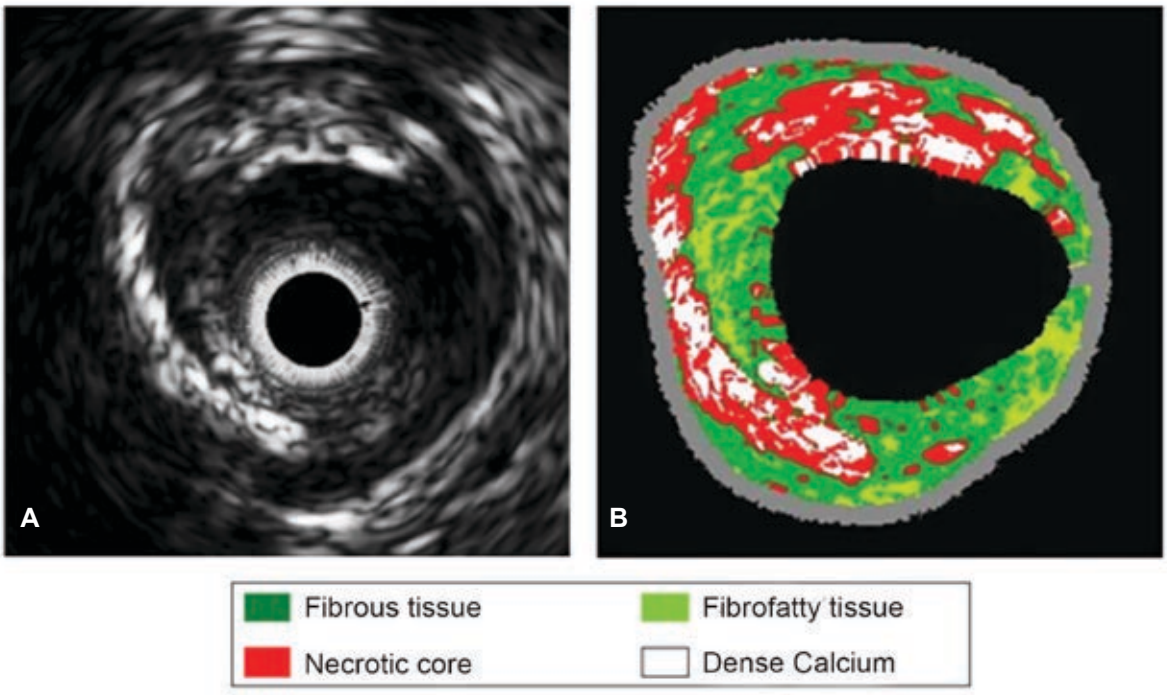


Figure 3. Automatic tissue characterization of the atheromatous plaque tissue components with IVUS-VH. A: Gray-scale IVUS shows a large eccentric plaque. B: The corresponding color-coded virtual histology image illustrates the tissue components of the atheromatous plaque. From König A, et al. Virtual histology. Heart. 2007; 93; 977-982, with permission from BMJ Publishing Group Ltd.

fatty tissue, 100% for necrotic core and 100% for calcified areas). However, these high rates were not reproducible in all studies. When IVUS-VH images were compared to plaques from porcine experimental models, the accuracy of tissue characterization did not exceed 60%.[17] It appeared that IVUS-VH often overestimated the presence of calcium. These discordances, although partly attributable to differences between human and porcine atherosclerotic pathogenesis, underscore the need for more studies of *in vivo* validation of IVUS-VH images. Another potential source of misclassifications is the presence of thrombus, which is colored as fibrous or fibrofatty tissue by IVUS-VH.[18] However, in practice, there are ways to distinguish thrombus from fibrofatty tissue, based on the clinical scenario (e.g. acute event versus stable patient with chronic coronary disease), the presence of necrotic core, the consistency of the tissue, etc.

As regards the reproducibility of morphometric and volumetric data obtained with IVUS-VH, it has been shown that reproducibility between different measurements by the same operator and between measurements by different operators is quite high: the correlation index for two different measurements on data acquired from one pullback by one operator was r=0.999, for measurements on data from two different pullbacks in the same vascular segment by the same operator r=0.997, and for data from two different operators in the same vascular segment r=0.995 ($p<0.001$).[19] The most reproducible data were those regarding the necrotic core.

Assessment of plaque vulnerability with IVUS-VH

Coronary thrombosis, which is the hallmark of acute coronary symptoms, has been correlated with three histologic substrates at the level of the atherosclerotic plaque: rupture, erosion and calcific nodules.[20] A specific type of atherosclerotic plaque, the thin cap fibroatheroma (TCFA), is considered as the histologic precursor of plaque rupture (it is, in essence, the histologic prototype of the vulnerable plaque) (Figure 4). TCFA is a plaque with a fibrous cap of 65 μm or less in thickness, infiltrated by macrophages and with a sizeable necrotic core.[20] IVUS-VH can be utilized for atherosclerotic plaque characterization, aiming at identifying a 'VH vulnerable plaque' or 'VH TCFA',

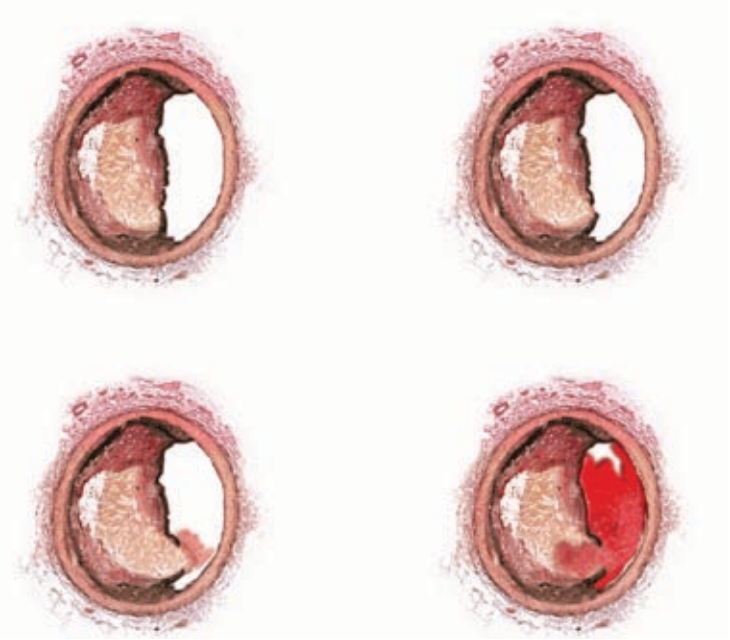


Figure 4. Top left: A vulnerable atheromatous plaque (thin-cap fibroatheroma: TCFA). Note the large necrotic core, separated only by a thin fibrous layer from the bloodstream. Top right: Initiation of cap rupture. Bottom left: Plaque rupture, contact of the necrotic core with blood elements and induction of thrombotic phenomena. Bottom right: Ruptured plaque, with thrombus formation and subtotal arterial lumen obstruction. Courtesy of Volcano Co.

corresponding to histologic TCFA. VH TCFA has been defined as a plaque rich in necrotic material (>10%) in contact with the lumen, taking up at least 40% of the arterial cross-sectional area (Figure 5).[21]

In addition to TCFA characterization, IVUS-VH may distinguish between various types of atheromatous plaque and identify specific vulnerability features (Figure 5). Such features, based on pathology studies, are the extent of necrotic core, the presence and thickness of a fibrous cap, the distribution of plaque calcification, the evolution of vascular remodeling, the degree of luminal obstruction and the location of the lesion. Information about these features obtained with IVUS-VH may be used to assess the vulnerability of the plaque. However, the clinical utility of such studies is in question, since it is doubtful that preemptive intervention on these plaques (minimally obstructive lesions with features of vulnerability) would in fact confer any benefit on the patient.[22]

The study of the vulnerable plaque and the determinants of its vulnerability has led to certain interesting characteristics of lesions especially prone to evolve from "vulnerable" to "culprit", i.e. giving rise to an acute coronary event. Data from IVUS-VH studies have contributed to the concept of "culprit of the culprit", i.e. the culprit part of a culprit lesion, which frequently does not coincide with the point of minimal lumen diameter and is rather found at the plaque borders (Figure 6A). These findings have obvious implications for the treatment of such lesions with stent implantation and render IVUS-VH a potentially very helpful technique for a more accurate choice of stent length, as well as stent positioning and deployment (Figure 6B). One should not fail to note, however, that these are still hypothetical assumptions, since clinical validation is lacking.

Studies of IVUS-VH clinical usefulness

Studies of prophylactic interventional treatment of minimally obstructive plaques have up to now been less than encouraging.[22] More studies with a head-to-head comparison of prophylactic intervention on high-risk plaques versus drug treatment are obviously needed. The PROSPECT study (Providing Regional Observations to Study Predictors of Events in the Coronary Tree) is a prospective study of the natural history of coronary artery disease, which will include imaging (including IVUS-VH).[23] Clinical follow up will last 5 years, all patients with coronary events will undergo coronary angiography, and the culprit lesion will be studied using intracoronary techniques. The findings will be compared to baseline coronary angiography and IVUS-VH data. The main purpose of the study will be to identify structural, morphological and geometrical parameters of the plaques, which could predict future events. Encouraging interim data from the PROSPECT trial were announced in the Transcatheter Cardiovascular Therapeutics (TCT) meeting in San Francisco in September 2009. Ac-

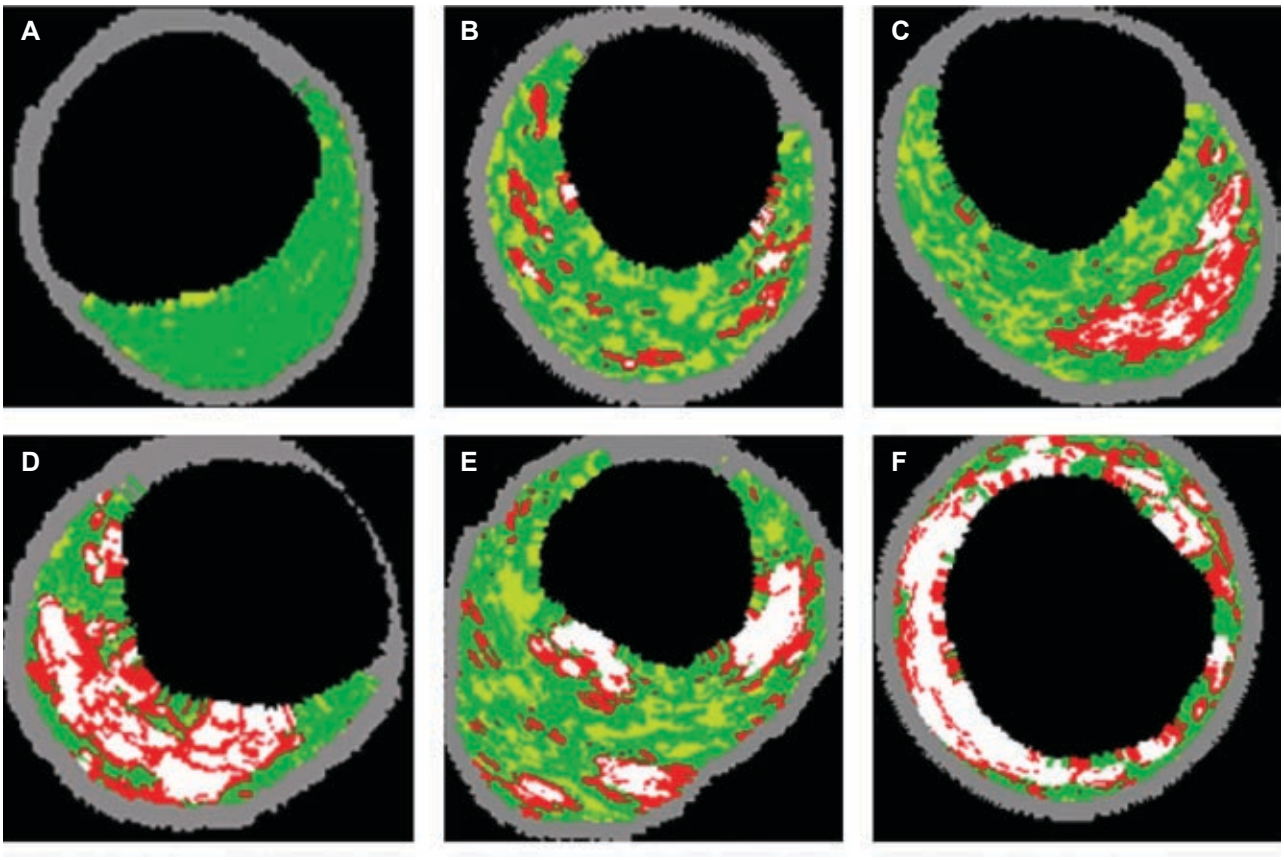
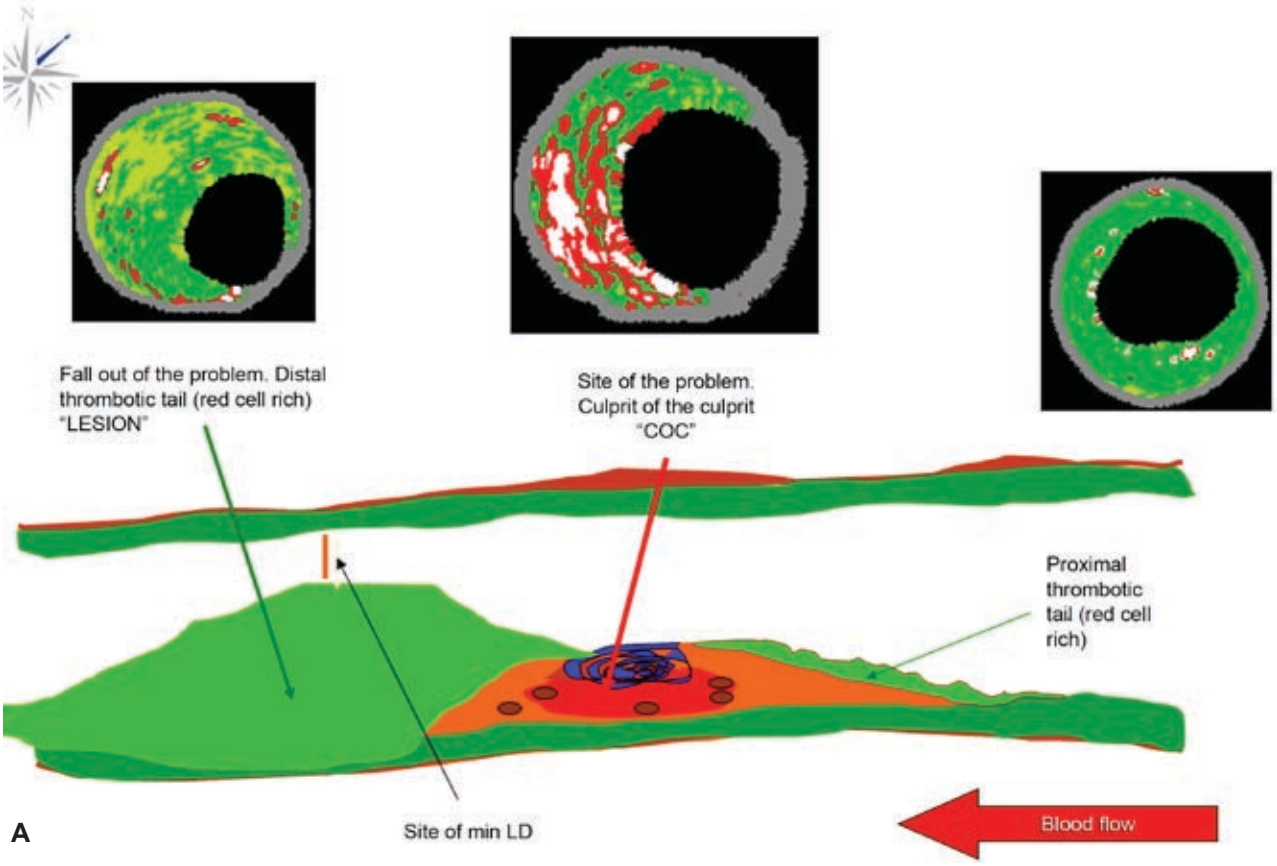


Figure 5. Classification of atheromatous plaques with IVUS-VH. A: Adaptive intimal thickening. B: Pathologic intimal thickening. C: Stable fibroatheroma. D: Thin-cap fibroatheroma (TCFA), according to the definition of TCFA with IVUS-VH: rich necrotic core in contact with the lumen. E: TCFA, with multiple layers of calcification and necrosis, suggesting multiple previous plaque ruptures. F: Fibrocalcific plaque. From König A, et al. Virtual histology. Heart. 2007; 93; 977-982, with permission from BMJ Publishing Group Ltd.

cording to these data, lesions with characteristics of "VH-TCFA" were significantly more likely to lead to a coronary event, compared to non-TCFA lesions (hazard ratio 3.84; p=0.001). These data, if confirmed by the final results of the study, are of particular importance, since they are the first to convincingly associate vulnerable plaque features with "hard" clinical events.

Another ongoing study is the privately funded Virtual Histology Global Registry, an international, multi-center registry of patients undergoing IVUS-VH.[24] Data from this registry have led to the first reports regarding the clinical utility of IVUS-VH. Missel et al[25] studied volumetric IVUS-VH data from 473 male patients and showed that the necrotic core to dense calcium ratio (NC/DC) was positively correlated with the total cholesterol to high density lipoprotein cholesterol ratio (TC/HDL-C) (r=0.18, p=0.0008), as well as with low density lipoprotein cholesterol (LDL-C) (r=0.17, p=0.002), while it was negatively correlated with HDL-C (r=-0.11, p=0.03). The NC/DC ratio was also higher in smokers than in non-smokers (p=0.006). The authors conclude that, since these lipidemic parameters and smoking are known risk-factors for sudden cardiac death, the NC/DC ratio, assessed by IVUS-VH, is potentially related to patient cardiovascular prognosis. In another study, Philipp et al[26] showed that IVUS-VH detects marked differences in coronary plaque composition related to traditional cardiovascular risk factors. Greater amounts of NC were associated with diabetes, hypertension, myocardial infarction and lower HDL-cholesterol levels. However, it should be noted that conclusions based on such surrogate markers of cardiovascular risk should be viewed with caution.

In an independent retrospective study, Hong et al[27] studied IVUS-VH plaque correlates to the no-reflow phenomenon in patients with acute coronary syndromes. In patients with no-reflow, in comparison to those not exhibiting no-reflow after intervention,



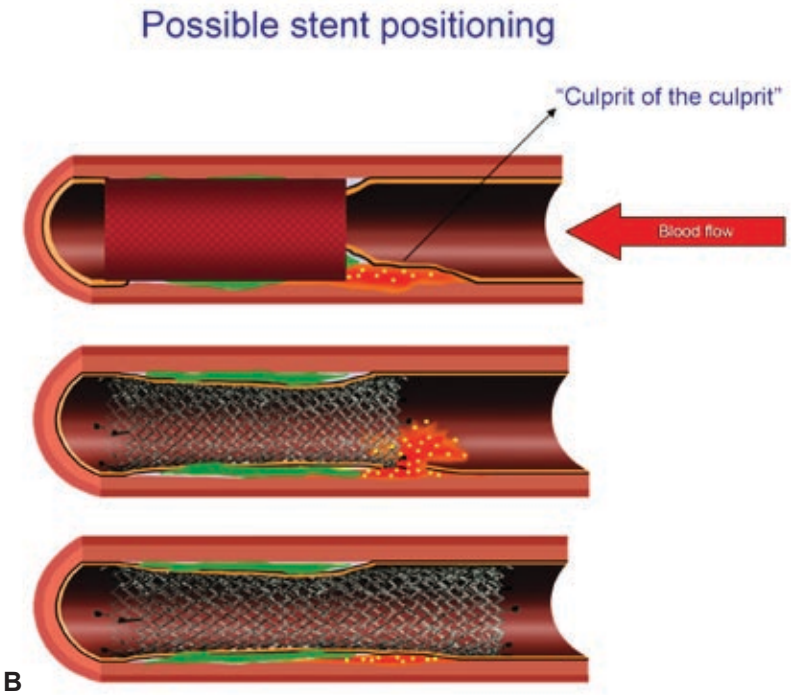


Figure 6. The "culprit of the culprit" notion, i.e. the culprit part of the culprit lesion, which may lie at the edge of the plaque, not corresponding to the site of minimal lumen diameter (A). (Courtesy of R Virmani and P Margolis, Volcano Corp.) This may be of particular importance for correct stent positioning and deployment (B). The use of the angiographic image alone may not be sufficient for optimal stent positioning. (Courtesy of Dr. Mark Wholey.)

necrotic core area was larger, both in absolute terms and as a proportion of the plaque volume (1.6 ± 1.2 mm^2 versus 0.9 ± 0.8 mm^2, $p<0.001$, and 24.5 ± 14.3% versus 16.1 ± 10.6%, $p=0.001$, respectively). Moreover, plaques with features of TCFA on IVUS-VH were more frequent in patients with no-reflow (71 versus 36%, $p=0.001$). In the multivariate analysis, the proportion of necrotic volume to total plaque volume was the only independent predictor of no-reflow (odds ratio 1.126, 95% confidence interval 1.045–1.214, $p=0.002$). In the same clinical setting, i.e. in patients with acute coronary syndromes, volumetric parameters of the culprit lesions have been associated with post-intervention myocardial damage. Uetani et al[28] report that the fibrofatty content of the plaque was positively correlated with post-intervention cardiac troponin T and creatine kinase-MB elevations. This correlation remained significant in the multivariate analysis.

More data are also needed with regard to IVUS-VH imaging of special situations, such as stented arterial segments. In an interesting study, Kim et al[29] underscore the difficulties of evaluating a stented segment. The authors studied sirolimus- or paclitaxel-eluting stents immediately after implantation and showed that most stent struts (80%) are erroneously identified by IVUS-VH as calcium (white) surrounded by necrotic area (red), while 17% of them are not visible at all (Figure 7). In the quantitative analysis, the presence of struts resulted in a (falsely) elevated proportion of dense calcium ($p<0.0001$) and necrotic core ($p<0.0001$). This pattern ("white areas with a red halo") (Figure 7) is obviously an artifact, which should be taken into account when stented segments are imaged with IVUS-VH.

Limitations of the technique

Despite the obvious advantages of the additional data provided by IVUS-VH regarding the structure of the atheromatous plaque and its potential correlation to clinical endpoints, there are certain limitations.

With regard to TCFA identification, it is of note that IVUS-VH axial resolution does not exceed 150 μm and its spatial resolution is even lower (240 μm),[14] while the histologic definition of TCFA includes a fibrous cap of 65 μm or less,[20] which means that IVUS-VH cannot accurately assess the thickness of the vulnerable fibroatheroma fibrous cap. Indeed, when "TCFAs" identified by IVUS-VH were evaluated with optical coherence tomography (OCT),[30] which has much higher resolution (10-20 μm), but less penetration (<2 mm), it was shown that 33 of the 61 "IVUS-VH TCFAs" did not truly have a thin fibrous cap (<65 μm). Conversely, 8 of the 33 "OCT TCFAs" were not truly rich in necrotic core (>10%) (misclassification by OCT due to low penetration). The advent of new, high-frequency IVUS catheters could increase IVUS-VH resolution, resolving this problem, at least in part. Another potential limitation is that IVUS-VH cannot identify cellular components of the vulnerable plaque, such as T-cells and macrophages (also included in the histologic definition of TCFA). These two facts denote that the vulnerable plaque identified by IVUS-VH is an entity not identical to the histologic TCFA; therefore, extrapolation of prognostic data gathered on histologically defined vulnerable plaques to IVUS-VH-identified TCFAs should be done with caution. Large longitudinal studies are needed to really assess the relation of IVUS-VH-identified features of vulnerability with future coronary events.

Although IVUS-VH can discriminate between some of the less echogenic components of the plaque (e.g. necrotic core and fibrofatty tissue), separating other soft plaque components, including thrombus, is not currently possible. Thrombus identification is important, as it could aid in identifying and assessing plaque ruptures in patients with acute coronary events. Moreover, shadowing caused by dense calcific areas can adversely affect correct identification of plaque components.[19]

Finally, it should be noted that plaque vulnerability is an evolving situation. The natural history of atheromatous plaques is virtually unknown; therefore, a plaque showing, at a given time point, features of vulnerability on IVUS-VH could have different characteristics if studied a few days later. Studies with serial *in vivo* IVUS-VH evaluations of atheromatous plaques are needed, in order to correctly outline the natural history of atheromatous lesions and their potential association with clinical events.

Conclusion

IVUS-VH is a promising emerging modality, offering the possibility to histologically characterize coronary atheromatous lesions. Technical drawbacks, mostly related to low resolution and misclassifications of histologic components, could be resolved to a significant extent with the advent of new, higher-frequency IVUS catheters, as well as with the continuous improvement of software, as data from *in vivo* studies are integrated in the design of histologic identification algorithms.

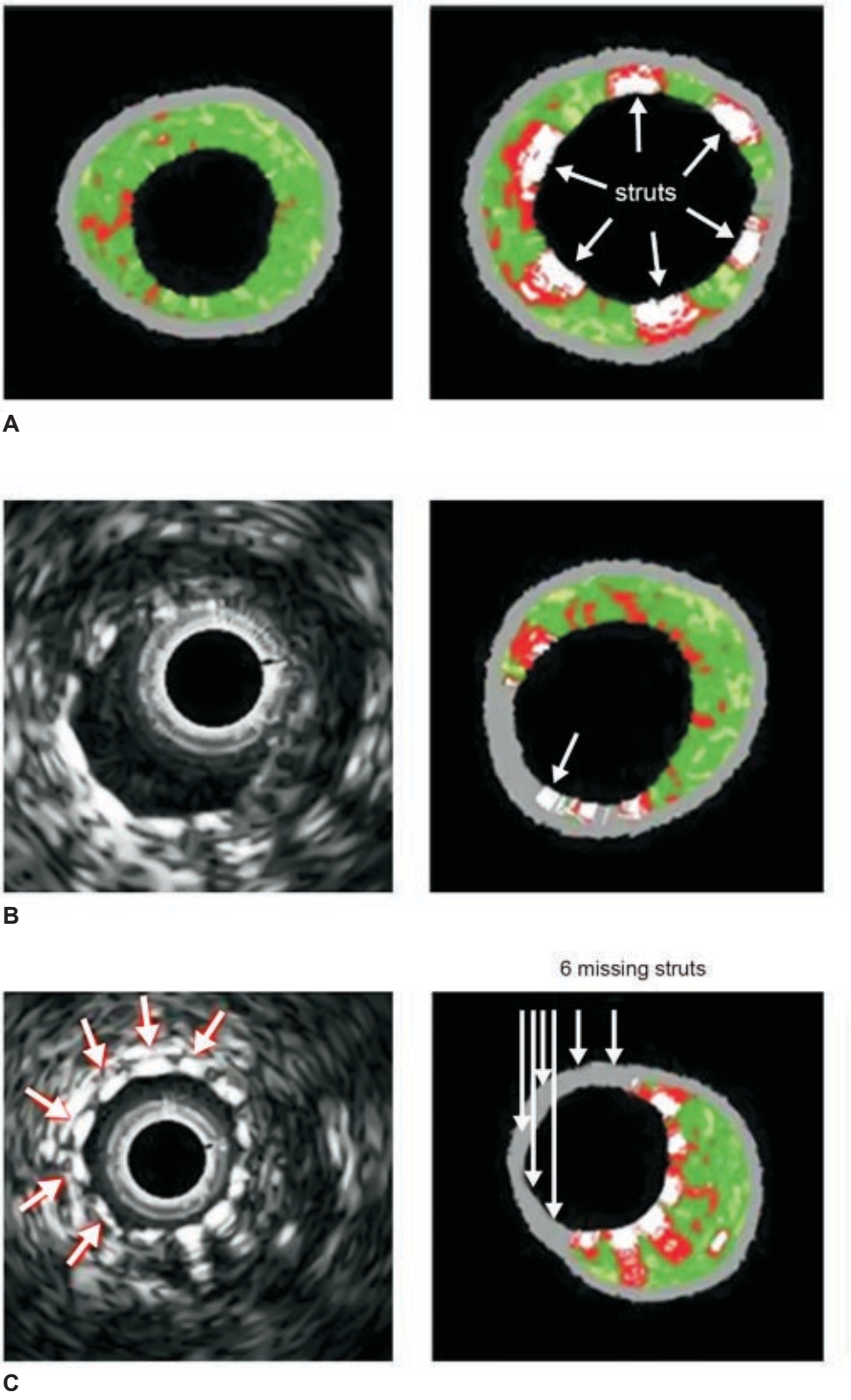


Figure 7. IVUS and IVUS-VH images before and after stent implantation. A: Stent struts (white color, i.e. identified as calcium), surrounded by a red halo (obviously an artifact). B: In this case, the strut appears white, without a red halo. C: Some of the struts are not imaged. From Kim et al. The virtual histology intravascular ultrasound appearance of newly placed drug-eluting stents. Am J Cardiol. 2008; 102: 1182-1186, with permission from Elsevier Ltd.

With regard to the clinical and prognostic value of IVUS-VH, data from large, prospective, multi-center trials are lacking. Small, preliminary published studies suggest that the features of atheromatous plaques, as identified by IVUS-VH, are correlated with epidemiological and clinical surrogate endpoints associated with prognosis, while interim results of ongoing clinical endpoint trials have associated IVUS-VH characteristics of coronary lesions with clinical events. However, further documentation of IVUS-VH practical applications, which could justify a broader employment of this modality in the catheterization laboratory, is still a matter for the future.

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