

Intravascular ultrasound radiofrequency analysis of coronary atherosclerosis: an emerging technology for the assessment of vulnerable plaque

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Evaluation of atherosclerotic plaque composition and morphometry may yield insight into plaque biology and the mechanisms of plaque-associated thrombosis. Analysis of intravascular ultrasound radiofrequency (IVUS-RF) backscatter signal is one technology that provides *in vivo* assessment of both atherosclerotic plaque composition and morphometry. We summarize three different approaches to IVUS-RF and critique the studies using this technology. In addition, we address the potential application of IVUS-RF to assess vulnerable plaque.

Introduction

Current therapies to reduce coronary events are targeted towards systemic risk factors. Identification of atherosclerotic plaque prone to thrombosis, i.e. vulnerable plaque, would allow for a refinement of risk prediction methods, potentially altering therapies. Analysis of intravascular ultrasound radiofrequency (IVUS-RF) backscatter is one method that characterizes plaque composition, potentially providing insight on the features of vulnerable plaque. We performed a systematic review of all studies found in the PubMed database published in English between 1983 and 2006 using the search terms of RF, IVUS, coronary artery disease, vulnerable plaque, integrated backscatter, wavelet analysis, and virtual histology. Two authors independently reviewed the relevant articles and abstracted data.

Intravascular ultrasound overview

IVUS is a catheter-based tool widely used to assess atherosclerotic burden.¹ At the tip of an IVUS catheter, a transducer emits an ultrasound signal and receives the reflected (backscattered) signal from tissue. In greyscale IVUS, the backscattered signal is processed in real-time into a two-dimensional video image. This image permits an accurate determination of vessel and lumen dimensions and the distribution, morphology, and severity of atherosclerotic plaque.² Visual, quantitative texture, and videodensitometric analysis of greyscale images have been used to measure plaque composition.^{3–5} However, none of these methods reproducibly discriminate elements of plaque

composition.^{3–8} This lack of accuracy may derive from a number of features of greyscale image analysis: reliance on video images which limits resolution to approximately 300 μm , operator-dependent parameters such as brightness and gain, and processing of the backscatter signal that distorts the relationship of the original acoustic data.⁹ Therefore, greyscale IVUS is a suboptimal tool to accurately and reproducibly identify plaque composition.

Analysis of radiofrequency data

RF data from the unprocessed backscattered ultrasound signal provides an alternative to greyscale image analysis. Theoretically, analysis of the IVUS-RF data provides a more accurate and reproducible technique for measuring tissue properties because it is not subject to machine-dependent processing or operator-dependent settings.^{10,11} Three different mathematical methods have been applied to RF data analysis including autoregressive modelling [IVUS-Virtual Histology™ (IVUS-VH), Volcano Corporation, Rancho Cordova, CA, USA], fast Fourier transformation (FFT) [Integrated Backscatter (IB-IVUS)], and wavelet analysis.^{11–13}

Autoregressive modelling of radiofrequency data—intravascular ultrasound-virtual histology

IVUS-VH categorizes atherosclerotic plaque into four distinct components that may be visualized on histological sections of coronary arteries stained with Movat pentachrome: (i) fibrous plaque that consists of densely packed collagen; (ii) fibro-fatty plaque comprised of collagen and interspersed lipid; (iii) calcified necrotic plaque that includes cholesterol clefts, foam cells, and micro-calcifications; and (iv) calcified plaque without adjacent necrosis.¹¹

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IVUS-RF data sets were constructed in a stepwise process. Ultrasound RF data was obtained from pullbacks on explanted human arteries. These arteries were sectioned and stained with Movat pentachrome stain. Homogeneous regions of interest (ROIs) for each one of the four plaque components were identified in these histological sections. The IVUS-RF data that spatially corresponded to the histological ROI were then analysed by autoregressive modelling. Autoregressive modelling converts RF data into a power spectrum graph that plots the magnitude of the backscattered ultrasound signal vs. the frequency.¹⁴ Fitting a linear regression line to this data permits identification of spectral parameters. Statistical classification trees sort the RF data based on combinations of these spectral parameters into one of four plaque components. RF data associated with a plaque component are assigned a colour code: green (fibrous), light-green (fibro-fatty), red (necrotic core), and white (dense calcium).

IVUS-VH tissue maps of explanted, atherosclerotic human arteries have a reported sensitivity, specificity, and predictive accuracy of 80–92% in identifying the four plaque types when compared with the corresponding histological sections of those arteries.¹¹ IVUS-VH tissue maps of coronary plaques generated by *in vivo* pullbacks have been compared with histological sections obtained from directional coronary atherectomy (DCA).¹⁵ Compared with histological exam of the DCA specimens, IVUS-VH identified plaque components with a predictive accuracy of 87% for fibrous, 87% for fibro-fatty, 88% for necrotic core, and 97% for dense calcium regions. However, comparisons between DCA specimens and IVUS-VH images are problematic, as DCA inherently disrupts tissue. Thus, mapping the IVUS-VH images to the corresponding histological ROI is very difficult and potentially inaccurate.

To overcome these potential inaccuracies, investigators have sought to assess IVUS-VH *in vivo*, using animal models of atherosclerosis. One recent study questions the accuracy of IVUS-VH in assessing plaque composition. Granada *et al.*¹⁶ assessed 60 lesions by IVUS-VH in an atherosclerotic porcine model. When compared with histology, IVUS-VH identified plaque components with an accuracy of 38–58%. In addition, IVUS-VH often misidentified calcium in the absence of histological calcification. The authors noted that differences exist between human and porcine atherosclerosis. However, the differences in atherosclerosis between these two species are subtle and would unlikely impact the broad classification of plaque components by IVUS-VH. Given these findings, we believe that continued *in vivo* validation studies are essential.

A critical step in employing IVUS-RF in longitudinal studies or clinical trials is to establish the reproducibility of IVUS-RF quantitation of vessel geometry and plaque composition. In one study, IVUS-VH data was acquired at 16 non-interventive lesions of 15 patients referred for elective percutaneous intervention (PCI).¹⁷ IVUS-VH interrogations were performed twice on each vessel and measurements were made by two blinded observers. Both inter-catheter and inter-observer differences were small (relative difference <1% inter-catheter and <5% inter-observer) for geometric measurements of vessel diameter and cross-sectional area (CSA). For measurements of plaque eccentricity, a value derived mathematically from plaque thickness, intra-observer and inter-observer differences were greater (relative difference of 5–10 and >50%, respectively). Inter-observer differences were <10% for calcium CSA and necrotic core, while the

inter-observer relative differences for fibrous and fibrolipidic CSA were greater (10 and 24%, respectively).

Integrated backscatter—intravascular ultrasound-radiofrequency data

FFT is another mathematical technique used in the analysis of IVUS-RF backscatter. FFT extracts frequency components of a signal buried in the original IVUS signal. Integrated backscatter (IB) is the average power of the FFT IVUS-RF backscatter signal from a small volume of tissue. IB values for the various plaque components can then be calculated to construct colour coded IB-IVUS tissue maps.¹²

IB-IVUS tissue maps were constructed from pullbacks on 18 coronary artery autopsy specimens.¹⁸ The sectioned arteries were stained with haematoxylin–eosin, elastic van Gieson, and Masson's trichrome and analysed histologically. Seven types of plaque composition were identified: calcification, fibrosis, lipid pool with fibrous cap, intimal hyperplasia, mixed lesions, media, and thrombus. Tissue maps were constructed by identifying IB values for ROIs within the histology sections that were homogenous for each of the tissue types. These tissue maps were tested against a separate set of ROIs from the same arteries. The authors stated that there was agreement between the IB-IVUS tissue maps and the actual histology, but they provided no data on sensitivity, specificity, or predictive accuracy.

A recent study evaluated the accuracy of IB-IVUS for tissue characterization of coronary plaques.¹⁹ IB-IVUS data was obtained from 42 coronary artery autopsy specimens. The IB-IVUS data was compared with 128 ROIs within the histological sections of the arteries stained with haematoxylin–eosin and Masson's trichrome. When compared with histology, the sensitivity of IB-IVUS for calcification, fibrosis, and lipid pool, was 100, 94, and 84%, respectively. However, the *in vivo* validation of IB-IVUS has been limited to comparisons with angiography, a relatively insensitive tool to assess plaque composition.¹⁸

Wavelet analysis of intravascular ultrasound-radiofrequency data

A wavelet is a waveform of limited duration and zero average amplitude. Wavelet analysis extracts a unique local wave pattern within a complex original IVUS-RF signal by iteratively computing wavelet coefficients that are localized by the amplitude and the position of wavelets. Theoretically, different ranges of wavelet coefficients correspond to specific plaque components, which would allow for plaque characterization.

The ability of wavelet analysis to detect lipid-laden plaque was evaluated in a small study of 27 explanted atherosclerotic arteries.¹³ Comparison of the wavelet data with the corresponding histological ROI, stained with Masson's trichrome stain, demonstrated a sensitivity of 83% and a specificity of 82% in detecting lipid-laden plaque. Similar *in vivo* comparisons of wavelet analysis with 29 plaques obtained from DCA also showed a high sensitivity (81%) and specificity (85%) for detecting lipid-laden plaque. The authors did not report whether wavelet analysis could identify other plaque components. We found no other studies that correlated wavelet analysis with non-lipid plaque components.

Limitations of intravascular ultrasound-radiofrequency validation studies

It is important to note that the current studies of the IVUS-RF modalities have significant methodological limitations: (i) the construction and validation of tissue maps for each IVUS-RF modality identified plaque components by Movat pentachrome stain or haematoxylin-eosin stain. Given the complexity of atherosclerotic plaque these tissue stains are subject to variable interpretation. A more thorough comparison of IVUS-RF tissue maps to histological sections would include specific stains for lipid, collagen, and calcium such as oil red O, birefringent picosirius red, and von Kossa's, respectively; (ii) a histological section and an IVUS-RF frame represent different plaque cross-sectional thickness. A histological section may be as thin as 4 μm . In contrast, all IVUS-RF data derives from an ultrasound beam whose width may be as great as 300 μm at its interface with the arterial wall. As a consequence, IVUS-RF is unlikely to detect subtle changes in plaque composition that occur over small distances;²⁰ (iii) the tissue maps of IVUS-VH and IB-IVUS and the wavelet coefficients were all constructed by comparisons with histological sections. Both plaque geometry and relative composition may have been subject to changes during the fixation, sectioning, and slide-mounting process. Only one small IB-IVUS study showed a significant correlation between IB-IVUS images taken immediately before and after fixation ($r = 0.87$; $P < 0.01$);¹⁸ (iv) the tissue maps of IB-IVUS or IVUS-VH and the wavelet coefficients constructed from *ex vivo* samples may not be representative of *in vivo* plaque. No study has tested the correlation of IVUS-RF data obtained *in vivo* to that obtained *ex vivo*. IVUS backscatter may be affected by blood, catheter movement, and cardiac contraction. This, in part, may be why IVUS-VH tissue maps obtained from *in vivo* interrogation of porcine arteries correlated poorly with histological sections of the same arteries;¹⁶ (v) validation studies of IVUS-RF that use DCA specimens are problematic, since the DCA tissue has been mechanically disrupted and exact spatial correlation may be difficult;^{11,13,15} (vi) the reproducibility of IVUS-RF data remains incompletely tested. The reproducibility of IVUS-VH has only been assessed in one small series,¹⁷ and there are no published data regarding the reproducibility of IB-IVUS or wavelet analysis; (vii) wavelet analysis has been used to identify lipid-laden plaque, which may be an advance over greyscale IVUS.¹³ However, wavelet analysis has not been shown to identify other plaque components; (viii) none of the IVUS-RF imaging platforms have been compared directly with one another. Therefore, it is not known which method, if any, is most accurate in characterizing plaque composition. The three IVUS-RF modalities clearly require further study to determine their accuracy in measuring plaque composition.

Intravascular ultrasound-radiofrequency assessment of atherosclerotic plaque

Insights into the development of plaque rupture and subsequent coronary thrombosis have predominantly arisen from necropsy studies in patients who experienced sudden cardiac death.^{21,22} In the majority of cases, the precipitant

was the development of an intraluminal coronary thrombus. Coronary thrombosis is associated with three plaque histologies: plaque rupture, plaque erosion, and calcific nodules.²³

Plaque rupture occurs in a majority of lesions that have overlying thrombi.²³ The histological precursor of plaque rupture has been designated the thin cap fibroatheroma (TCFA), defined as a lesion with a fibrous cap $< 65 \mu\text{m}$ thick, infiltrated with macrophages, with a well developed necrotic core.²³ Investigators have used IVUS-VH to define a surrogate for histological TCFA, termed IVUS-derived TCFA. One study performed an analysis to detect TCFA with IVUS-VH in 55 patients.²⁴ IVUS-derived TCFA was defined as the presence of a focal, necrotic-core rich ($> 10\%$) plaque in contact with the lumen with a percent atheroma CSA $> 40\%$ (Figure 1). The investigators determined that IVUS-derived TCFA was more prevalent in acute coronary syndrome (ACS) patients and occurred more commonly in the proximal segments of arteries. A potential limitation of this study is the axial resolution and spatial accuracy of IVUS-VH. IVUS-VH has an axial resolution of 150 μm and spatial accuracy of 240 μm ,¹¹ whereas the accepted histological definition for TCFA requires a thinner fibrous cap.²³ Moreover, IVUS-VH cannot visualize cellular components such as T cells and macrophages, both features of histological TCFA. Clearly, IVUS-derived TCFA is an entity distinct from histological TCFA, and its importance will need to be assessed in longitudinal studies.

Expansive arterial remodelling, defined as an enlargement of the external elastic membrane (EEM) CSA in the presence of atheroma, is also a component of unstable plaques.²⁵ Greyscale IVUS studies have shown that culprit lesions in ACS occur more often in areas of expansive remodelling.²⁶ On the basis of these findings, investigators have used IVUS-VH to assess plaque composition in areas of expansive

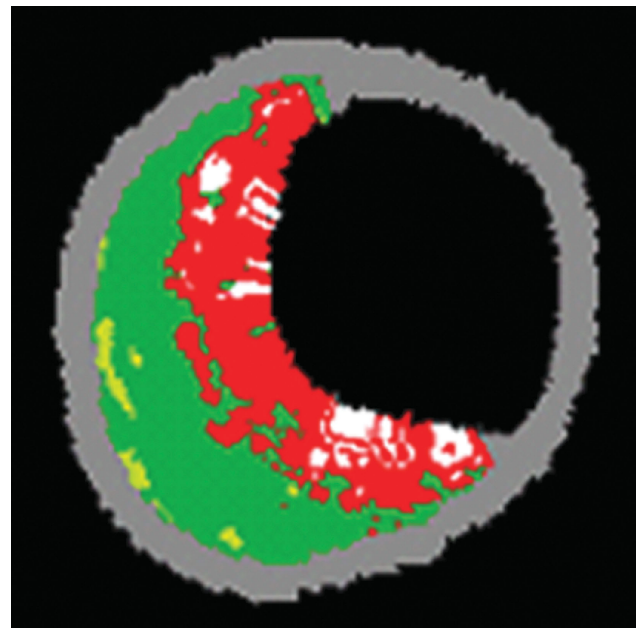


Figure 1 Intravascular ultrasound-derived thin cap fibroatheroma. Compositional analysis identified plaque burden: 67%, necrotic core (red): 39%, fibrous (green): 51%, fibro-fatty (yellow): 3%, and calcific (white): 8%. The lack of fibrous tissue overlying the necrotic core is noteworthy, which is in direct communication with the lumen.

remodelling.^{27,28} Lesions with expansive remodelling had a larger necrotic core and less fibrous tissue when compared with non-remodelled lesions or lesions with constrictive remodelling (decrease in the EEM CSA). Lesions with expansive remodelling were predominantly composed of fibrofatty tissue (fibroatheromatous) or IVUS-derived TCFA. In contrast, only a small number of lesions with constrictive remodelling were fibroatheromatous and none were IVUS-derived TCFA.²⁷ These cross-sectional studies suggest that atherosclerotic lesions with expansive remodelling have different plaque composition than lesions with constrictive remodelling. It remains unknown whether these compositional differences are predictive of thrombotic events.

Epidemiological studies demonstrate that culprit lesions in ST-segment elevation myocardial infarction are prevalent in the proximal third of coronary arteries.²⁹ Valgimigli *et al.*³⁰ performed an IVUS-VH study to investigate whether plaque location within the coronary tree influenced plaque composition. Fifty-one patients with stable angina underwent IVUS-VH of a non-culprit artery. The overall ROI was divided into 10 mm segments. No differences between segments were observed in the amount of fibrous, fibro-fatty, or calcific tissue. There was an increase in lipid content in the first 10 mm segment closest to the ostium when compared with segments of 30 and 40 mm distal to the ostium. In multivariable regression analysis, the distance from the ostium was an independent predictor of lipid content. The findings of this small study suggest a non-uniform distribution of necrotic content along coronary arteries and need to be confirmed by a larger study.

Autopsy studies suggest that the majority of occlusive intracoronary thrombotic events arise from plaque rupture. Therefore, assessment of ruptured plaque may provide insight into the features of vulnerable plaque. Rodriguez-Granillo *et al.*³¹ recently described IVUS-VH-derived plaque composition in lesions with plaque rupture. Plaque rupture was defined as 'a ruptured capsule with an underlying cavity or plaque excavation by atheromatous extrusion with no visible capsule'. Forty patients with ACS underwent three-vessel IVUS. Plaque rupture occurred in 28 lesions (20 patients). Though widely distributed throughout the circumflex and right coronary arteries, the greatest proportion of plaque rupture was in the left anterior descending coronary artery. Ruptured lesions compared with lesions without plaque rupture had similar plaque burden but a larger vessel CSA, findings indicative of greater expansive remodelling in ruptured lesions. Ruptured plaque also had a greater percentage of necrotic core. Although limited by its small sample size and the use of multiple comparisons, this study is consistent with earlier reports that linked expansive remodelling with coronary events. The study also reveals a correlation between necrotic core and plaque rupture.

The ideal method to investigate vulnerable plaque is through longitudinal patient evaluation. Sano *et al.*³² used IB-IVUS to identify tissue characteristics of atherosclerotic plaques prior to the development of ACS. One hundred and forty patients with angina were enrolled. During longitudinal observation, 12 patients had ACS and an angiographically identified culprit lesion. IB-IVUS data had been obtained on 10 of these 12 plaques during initial enrolment. These 10 plaques were classified as vulnerable plaques, whereas

the remaining lesions were deemed stable plaques. In comparison with stable plaques, vulnerable plaques had greater plaque burden, eccentricity, increased remodelling index, larger percentage of lipid area, and decreased fibrous area. Although limited by the small number of patients who developed ACS and the lack of IB-IVUS interrogation at follow-up, this study is the only one that has used IVUS-RF to assess plaques that progressed to thrombosis.

The same investigators assessed the effect of statin therapy on plaque composition using serial IB-IVUS interrogation.³³ Fifty-two patients with stable angina and hyperlipidaemia were assessed with IB-IVUS and randomized to statin or diet therapy, and reassessed by IB-IVUS in 6 months. Statin therapy was associated with a decrease in lipid component and no significant change in plaque area, although the 6-month follow-up period may have been too short to observe plaque regression. Previous studies such as ASTEROID and REVERSAL have shown that intensive statin therapy leads to a reduction in plaque volume, as assessed by serial greyscale IVUS.^{34,35} These IB-IVUS results potentially extend upon those findings, suggesting that statin therapy, in addition to plaque regression, may alter plaque composition.

In aggregate, these early IVUS-RF studies provide preliminary data, which serve to refine hypotheses regarding vulnerable plaque. Prospective clinical studies with longitudinal follow-up will confirm or refute the importance of defining plaque composition by any of the IVUS-RF modalities.

Clinical validation of intravascular ultrasound-radiofrequency

IVUS-RF requires rigorous clinical validation. Two endeavours are underway that will help elucidate the utility of IVUS-RF. The Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) trial is a prospective study, which has enrolled 700 patients with ACS.³⁶ Eligible patients must have one- or two-vessel coronary disease requiring PCI. All three coronary arteries will be assessed by quantitative angiography, greyscale IVUS, palpography, and IVUS-VH. Clinical follow-up is scheduled annually for 5 years or is event-driven. All patients with repeat events will undergo repeat angiography to identify the culprit lesion. The site of the culprit lesion may then be matched to the original angiogram and IVUS-VH data in order to identify plaque composition and morphometric features that may predict events. The other study is the Virtual Histology Global Registry, an industry sponsored, international, multicenter registry of 2000 patients who have undergone IVUS-VH. This cross-sectional study will provide plaque compositional data across multiple patient subgroups. Data from these studies will help provide the required framework to determine the clinical utility of IVUS-VH.

Limitations of intravascular ultrasound-radiofrequency

Each of the three modalities of IVUS-RF (IVUS-VH, IB-IVUS, and wavelet analysis) still requires substantial validation work before its application in diagnosis and therapy in

patients with atherosclerotic disease. First, the accuracy of each modality in assessing atherosclerotic plaque composition requires more rigorous assessment. Once the accuracy of IVUS-RF is proven, then the relative merit of IVUS-RF will need to be compared with other non-invasive and invasive technologies that assess atherosclerotic plaque (computed tomography angiography, magnetic resonance imaging, radionuclide imaging, intracoronary thermography, intravascular palpography, optical coherence tomography, near-infrared spectroscopy, Raman spectroscopy). Finally, the objective of plaque composition is to improve the detection of vulnerable plaque, which itself is a controversial concept. Although histological examination of coronary arteries from victims of sudden cardiac death have shown that TCFA is commonly associated with plaque rupture,²³ the significance of TCFA in living subjects is unknown. Evidence of multiple disrupted plaques and widespread inflammation throughout the vascular bed in some patients with ACS,^{37,38} suggests that it may be more appropriate to focus attention at a systemic level, i.e. towards the vulnerable patient. Ultimately, the results of large, prospective studies with long-term follow-up will determine whether the identification of vulnerable plaque by any IVUS-RF modality is diagnostically and therapeutically useful.

Conclusions

Analysis of IVUS-RF backscatter signal permits geometric measurements and quantitative compositional analysis of atherosclerotic plaque. Thus, IVUS-RF potentially improves upon greyscale IVUS. However, IVUS-RF techniques have been incompletely validated in their ability to determine plaque composition. Small clinical studies suggest that analysis of both plaque geometry and composition by IVUS-RF improves the ability to predict which plaques lead to coronary thrombosis. Continued validation of IVUS-RF, coupled with longitudinal clinical trials, will determine whether IVUS-RF improves the approach to patients at risk of coronary thrombosis.

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