Virtual Histology-Intravascular Ultrasound as a diagnostic alternative for morphological characterization of carotid plaque: comparison with histology and High-Resolution Magnetic Resonance findings

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Purpose

Stroke is the third most common death cause and one of the most common cause of long-term disability in the western world [1]. Carotid plaque morphology is, more than the stenosis degree, the main predictor of cerebro-vascular accidents. Presence of ulcerated or ruptured plaque is a common morphological aspect of symptomatic lesions [2-3].

Several histological studies have demonstrated that atherosclerotic plaques associated to cerebral ischemia contain an abundant lipid core with a thin fibrous cap [4]. According to a study by Yuan et al, correlating clinical symptoms to carotid artery plaque composition assessed by MR, patients with a fissured fibrous cap appear to have a 23-fold increased risk of developing TIA or stroke compared to patients with a thin but intact fibrous cap [5].

Improvements in knowledge about histological and evolutional aspects of carotid artery plaques raised the need for plaque characterization, in order to determine the most adequate procedure and devices for stroke prevention.

High-Resolution Magnetic Resonance (HR-MR) Imaging demonstrated to be the most sensitive and specific diagnostic tool for in vivo morphological characterization of atheromatous carotid artery plaques [6-7], strongly in agreement with histology [1].

Virtual Histology- IntraVascular UltraSound (VH-IVUS) may be a valid diagnostic alternative for the in vivo morphological characterization of carotid artery plaques, whenever a pre-procedural HR-MR may not be performed, for a "real time" choice of the devices or to monitor high risk embolization plaque during stenting procedure [8].

Primary target of this study was, thus, to validate the use of VH-IVUS in the ex vivo characterization of carotid artery plaques by correlation to histological specimen. Secondary target was to compare in vivo VH-IVUS with HR-MR Imaging in terms of the precision rate (positive predictive value) for plaque characterization.

Methods and Materials

Ex vivo VH-IVUS - Histology correlation

Patients

Data was acquired from six human carotid arteries explanted previous post mortem familial consent and after approval from our hospital's ethic committee.
Six single carotid arteries were explanted from six consecutive male patients with a mean age of 72.5 ± 5.79 years, with known history of cerebral ischemia, either Transient Ischemic Attack (TIA) or stroke [Table 1].

Patients with a previous endovascular or surgical revascularization were excluded.

*Ex vivo specimen preparation*

The time interval between death and specimen explantation ranged from 8 to 12 hours. VH-IVUS imaging was performed within 48 hours from death. The arteries were explanted from the common carotid artery ostium to the distal extra cranial segment, including approximately 40 mm of the surrounding adipose and muscular tissues in order to maintain a supporting structure for the vessels, fixated in a 10% formalin solution and conserved at room temperature before the study.

Specimens were mounted on the *dissecting tray*, after being filled with a 10% formalin or saline solution. A compressed air system, designed at the Biophysics Department of Tor Vergata University, was used to allow phosphate buffered saline (PBS) flow through the vessels. The arterial branches were clamped to allow an intraluminal pressure of 100 mmHg, as described in other studies [9-10]

*IVUS and histological data acquisition*

Ultrasonographic data was acquired using a 20 MHz IVUS apparatus (Eagle Eye, Volcano Therapeutics, Rancho Cordova, CA). After introducing the IVUS catheter into each artery, sectional images of the plaques were obtained using a 1.0 mm/sec *pull-back* system. For this study, an area of stenosis > 60% for each section evaluated by IVUS was considered as significant.

VH-IVUS data was successively stored on a personal computer for further off-line analyses. Each specimen was then immediately immersed in saline solution and conserved in sealed 15-20 ml vials for the 2 hours following the IVUS session. The specimens were maintained at room temperature to avoid a phase transition of the constituents. A *quick-freeze* of the vials was not performed in order to guarantee the integrity of the specimen and to avoid a distortion of the tissues caused by the presence of internal calcifications.

The carotid artery specimens were fixated in buffered 10% formalin solution for 24 hours. The methods for specimen preparation have been previously described [11-12].

Each section was numbered sequentially from the proximal to the distal segment of the internal carotid artery in order to reconstruct the plaque in its whole length.

*Histological Analysis*
Two histopathologists analyzed the histological sections, unaware of the results obtained by VH-IVUS, at first independently and then jointly, to rule out interpretation doubts. Kendall's W Test was used to assess the agreement among the operators. The analysis was performed according to the classification of atherosclerotic lesions of the American Heart Association (AHA) Council on Atherosclerosis [13]. Four types of plaque components were identified: collagen, fibrolipid core, calcium and necrotic tissue. Areas with predominant collagen content were assimilated to fibrous tissue, while areas with lipid content predominating over collagen to fibro-lipid tissue. Regions containing residues of cholesterol, foam cells and micro-calcifications were defined as necrosis. After identifying the different areas within the plaque, digital images were created (72dpi, dot per inch) using an artwork software (Adobe Photoshop version CS3, Adobe Systems Inc., CA, USA) setting a specific color to each of the four tissues considered.

The number of pixels (picture elements) of each of the four colors was calculated and, accordingly, the percentage of the different tissue components was defined in all plaques. The percentages obtained were considered as the reference standard for the study.

**VH-IVUS - Histology correlation**

The exact correspondence between the histological sections and the VH-IVUS images was determined using the sutures which were placed on the outer surface of each artery, using the morphology of each section to obtain an acceptable section match.

The VH-IVUS and the digitalized histopathological images (png format) were optimized to raster images and normalized to a standard dpi format (72 dpi).

The VH-IVUS slices used for the analysis (8-10 per specimen) were selected accordingly to the existence of regions involved by critical plaque that were identified on the digitalized images by an expert histopathologist.

Using a pixel-by-pixel segmentation, the VH-IVUS Lab software (Volcano Therapeutics Inc.) elaborated 4 tissue maps for each selected image: fibrous tissue, fibro-lipid tissue, necrosis and calcium.

The selected digital histopathological images were then processed through their manual segmentation into four different tissue segments by an expert histopathologist unaware of the VH-IVUS findings, using an artwork software (Adobe Photoshop, version CS3 Adobe Systems Inc., CA, USA) [Fig.1]. Finally, the percentage of each tissue component was defined according to the number of pixels contained in the different segments [14].

Forty-two of the 54 images obtained were evaluated of satisfactory quality, 12 were discarded because of either incomplete or jeopardized data.

**In-vivo VH-IVUS - HR-MRI correlation**
Patients

Twelve consecutive patients (8 males, 4 females, mean age of 75 ± 6.33 years), candidates for Carotid Artery Stenting were included in this study. The enrolled patients were considered symptomatic after neurological evaluation. All of them underwent MRI brain examination with intracerebral angiographic sequences within three month from the last clinical symptom.

The degree of the stenosis ranged between 60 and 80 % and were identified by eco-color-Doppler ultrasound and Computed Tomography Angiography. All patients had undergone a neurological evaluation and a brain CT or MR scan before endovascular treatment.

In-vivo HR-MRI

The stenoses were localized by Duplex Doppler Ultrasound ultrasonography and their exact position was localized on the skin to allow a precise positioning of the MR microcoil. Patients were evaluated within 15 hours before the endovascular procedure.

Exclusion criteria consisted in the presence of general contraindications to MR or to gadolinium contrast medium administration.

The HR-MRI study protocol was performed using a 1.5 Tesla apparatus (Gyrosan Intera, Philips, Best, The Netherlands), with a maximum gradient strength and slew rate of respectively 33mT/m and 80 mT/m/ms), and a microscopy radiofrequency surface coil (Microscopy 47mm, Philips, Best, The Netherlands) positioned at the carotid bifurcation. Patients were examined in supine position using a head support in order to reduce movement artifacts. The head was slightly turned on the opposite side of the examined carotid artery to expose the carotid bifurcation, and to prevent sterical constraints from the mandibular bone and sternocleidomastoid muscle.

Acquisitions were triggered with the heart frequency, which was monitored during the whole examination using an electrocardiograph (cardiographic gating VCG), in order to reduce artifacts related to arterial sphygmic movements.

Total examination time, including patient positioning, was approximately 40-45 minutes.

VH-IVUS Imaging protocol and Carotid Artery Stenting (CAS)

An informed patient consent was obtained for each patient prior to the procedure. A 5-day anti-aggregation therapy with acetilsalicilic acid (100 mg / day) and clopidogrel (75 mg / day) (Plavix® - Bristol-Myers Squibb/Sanofi Pharmaceuticals Partnership Bridgewater) was administered before the procedure.
In each procedure, a bolus of 5000 IU of heparin was administered intravenously to maintain the Active Coagulation Time (ACT) between 200 and 250 seconds after obtaining transfemoral retrograde access. During each procedure, a cerebral protection device (Epi-filter, Boston Scientific) was placed before VH-IVUS examination and stent deployment. The Gray-Scale and VH-IVUS evaluations were performed using a 20-MHz IVUS (EagleEye, VolcanoTherapeutics, RanchoCordova, CA) probe. The IVUS catheter was washed with saline solution prior to its use. The internal carotid artery was selectively catheterized using the IVUS catheter. After optimization of the gain, the IVUS catheter was retrieved from the distal segment of the internal carotid artery at a speed of 1.0 mm/sec using a motorized pull-back device. The IVUS scan during the pull-back maneuver included at least one proximal (vessel ostium) and one distal marker to allow a comparison between the VH-IVUS and MR images. Examinations were recorded on a DVD.

Each patient underwent a brain MR scan 2 hours after CAS.

**HR-MR and VH-IVUS data correlation**

Two expert radiologists determined the adequacy of the selected images for the correlation, applying the criteria of the *American College of Cardiology/American Heart Association (ACC/AHA) consensus statement* on IVUS (External Elastic Membrane - EEM visible for at least 270°) [15]. Both radiologists evaluated the presence of fibro-lipid tissue, fibrous tissue, necrosis or calcium in the plaque, first independently and then jointly in order to rule out eventual interpretational doubts.

The specific tissue components were defined as follows: fibro-lipid tissue as a relatively hypoechoic area compared to the adventitia, fibrous tissue as a hyperechoic area without acoustic shadow and calcium as a hyperechoic area with acoustic shadow. Afterwards, the manual elaboration of the margins of each axial image was performed to obtain VH images.

The intra-luminal margin and the EEM were then identified. The two radiologist then selected the images for the VH-IVUS/HR-MR correlation. The correspondence between the images of the two methods was determined according to the distance from the vessel's ostium and the presence of identical morphological characteristics. Evaluating the morphology of the plaque and using the jugular vein as an orientation landmark, correct orientation and overlapping between the VH-IVUS and HR-MR images was achieved.

All HR-MR images were converted to a digital format (tiff) using a dedicated console, preserving the original signal intensity of the pixels. The exact match between VH-IVUS and HR-MR images was obtained using as a reference the distance from the vessel ostium and the intrinsic morphological characteristics of the vessel sections. The exact position of the IVUS probe, visible also on the DSA images due to its radiopacity,
was determined according to the known speed (1.0 mm/sec) of the motorized pull-back system. HR-MR and VH-IVUS images with higher atheromatous involvement were selected for the correlation analysis.

Selected HR-MR and VH-IVUS images, optimized to raster graphics images and converted to a standard tiff format (72 dpi), were examined by both radiologists with the same criteria used for VH-IVUS-imaging processing, using a pixel by pixel segmentation. Images were merged into four tissue classes: fibrous tissue, fibro-lipid tissue, necrosis and calcium using the same method used for the histopathological images, as previously described. These classes were correlated to the plaque components identified by VH-IVUS: fibrous tissue (green), fibro-lipid tissue (yellow), necrosis (red) and calcium (white). To establish the presence of a specific plaque component, multiple adjacent pixels had to show the same signal intensity.

Once digital images were obtained and the number of pixels for each plaque component was calculated, the exact percentages of the 4 plaque components were determined [Fig.2].

**Statistical analysis**

The percentages of the various plaque components provided by the observers were compared for each method.

Agreements between the diagnostic methods results were estimated using the Cohen's k test\[16\] and its relative 95% confidence interval (95% CI). Specificity, sensitivity, precision rate (positive predictive value), concordance and the 95% CI of the VH-IVUS versus Histology arm of the study were estimated considering the Histology result as the gold standard. A k value included in a range of 70% to 85% was considered as good agreement, a value included in a range of 86% to 100% agreement was considered as excellent inter-observer agreement\[17\]. Specificity, sensitivity, precision rate (positive predictive value), concordance and the 95% CI of the VH-IVUS versus HR-MR arm of the study were estimated considering the HR-MR result as the gold standard. Significance was assessed at 5% level. The statistical software package used for this analysis was SPSS for Windows (version 17.0; SPSS Inc., Chicago IL, USA).

All statistical analyses were performed by two authors and a statistician.

**Images for this section:**
**Fig. 1:** Fig. 1 (a) Histological image. (b-d) Digitalized image processed through its manual segmentation into four different tissue segments by an expert histopathologist. Brown, external elastic membrane; yellow, fibro-lipid tissue; green, fibrous tissue; white, calcium; red, necrosis. (c) VH-IVUS showed a good correlation with histology especially with regards to the differentiation of the fibrous and fibrolipid tissues.
Fig. 2: Fig. 2 (a) Fibrolipid plaque with interruption of the fibrous cap reported on the PDw and (b) T2 image, with a small ulceration, that can be easily seen on the corresponding
virtual histology (VH) image. (c) The T1w GD sequences show enhancement of fibrous cap, mainly in the site of the rupture. (d, e) The normalized magnetic resonance image shows significant correlation with the VH findings.

Table 1: Key baseline features of ex-vivo patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Comorbidities</th>
<th>Stenosis degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73</td>
<td>Male</td>
<td>Hypertension</td>
<td>71%</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>Male</td>
<td>Type II DM</td>
<td>80%</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>Male</td>
<td>COPD, hypertension, smoker</td>
<td>78%</td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>Male</td>
<td>Hypercholesterolemia</td>
<td>70%</td>
</tr>
<tr>
<td>5</td>
<td>71</td>
<td>Male</td>
<td>Hypertension, chronic heart failure</td>
<td>67%</td>
</tr>
<tr>
<td>6</td>
<td>73</td>
<td>Male</td>
<td>COPD, smoker</td>
<td>69%</td>
</tr>
</tbody>
</table>

Table 2: Virtual histology-intravascular ultrasound: histology correlation

<table>
<thead>
<tr>
<th>VH IVUS ex-vivo accuracy (histology as reference)(^a)</th>
<th>Precision rate</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrous tissue</td>
<td>99.4% ± 0.6</td>
<td>98.7% ± 1.2</td>
<td>96.6% ± 1.7</td>
</tr>
<tr>
<td>Fibrolipidic tissue</td>
<td>85.9% ± 3.1</td>
<td>94.2% ± 2.3</td>
<td>84.3% ± 4.4</td>
</tr>
<tr>
<td>Calcium</td>
<td>71.4% ± 15.2</td>
<td>84.1% ± 3.33</td>
<td>97.4% ± 1.9</td>
</tr>
<tr>
<td>Necrosis</td>
<td>83.4% ± 6.4</td>
<td>67.1% ± 9.7</td>
<td>99.2% ± 0.5</td>
</tr>
</tbody>
</table>

VH-IVUS, virtual histology intravascular ultrasound. \(^a\) Overall concordance: \(k = 82\%\) (95% confidence interval 69–92%).

Table 2: Table 2 Virtual histology-intravascular ultrasound: histology correlation
<table>
<thead>
<tr>
<th></th>
<th>Precision rate</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrous tissue</td>
<td>85.3% ± 8.1</td>
<td>86.1% ± 6.1</td>
<td>84.3% ± 7.6</td>
</tr>
<tr>
<td>Fibrolipidic tissue</td>
<td>95.2% ± 2.8</td>
<td>93.7% ± 4.2</td>
<td>97.5% ± 1.3</td>
</tr>
<tr>
<td>Calcium</td>
<td>90.2% ± 5.33</td>
<td>89.3% ± 3.9</td>
<td>99.2% ± 0.4</td>
</tr>
<tr>
<td>Necrosis</td>
<td>82.0% ± 9.6</td>
<td>65.4% ± 18.6</td>
<td>98.1% ± 1.1</td>
</tr>
</tbody>
</table>

HR-MR, high-resolution magnetic resonance; VH-IVUS, virtual histology intravascular ultrasound. 

*Overall Concordance: k = 84% (95% confidence interval 67 – 90%).*

**Table 3:** Table 3 High-resolution magnetic resonance: virtual histology intravascular ultrasound correlation
Results

Ex vivo VH IVUS - Histology correlation

Forty-two images of the 54 ex vivo VH-IVUS images obtained were used for correlation (8-10 sectional images obtained by 6 explanted carotid arteries) with histology. Twelve images were excluded either due to incomplete or jeopardized VH-IVUS data. The agreement between the two histopathologists, calculated using the Kendall's W test, resulted in a W of 0.91.

Sensitivity of VH-IVUS in characterizing fibrous tissue, fibro-lipid tissue, calcium and necrosis, considering histology as the reference standard, resulted respectively 98.7%, 94.2%, 84.1%, and 67.1%, while its specificity was respectively 96.6%, 84.3%, 97.4%, and 99.2%.

Precision rate of VH-IVUS with true histology of different plaque components resulted 99% for fibrous tissue, 86% for fibro-lipid tissue, 71% for calcium and 83% for necrosis [Table 2].

Overall concordance between VH-IVUS and Histology resulted good 82% (95% CI 69% to 92%).

No significant association between VH-IVUS precision rate and stenosis degree was observed in the ex vivo arm of the study.

In vivo HR-MRI - VH-IVUS correlation

During in-vivo IVUS evaluation no clinical complications were observed. No major or minor cerebrovascular ischemic events occurred in clinical and instrumental examination after CAS procedure.

In all cases it was possible to obtain a complete characterization of plaque morphology.

Comparison between HR-MR and VH-IVUS was performed on 27 images. Sensitivity of VH-IVUS in the characterization of fibrous tissue, fibro-lipid tissue, calcium and necrosis, considering HR-MR as the reference standard, resulted respectively 86.1%, 93.7%, 89.3% and 65.4%; specificity resulted respectively 84.3%, 97.5%, 99.2%, and 98.1%.

Precision rate resulted respectively 85% for fibrous tissue, 95% for fibro-lipid tissue, 90% for calcium and 82% for necrosis, [Table 3]. In 6 images, HR-MR identified areas of contrast-enhancement in the fibrous cap.

Overall concordance between VH-IVUS and HR-MR resulted good (84%) between the two methods (95% CI 67% to 90%).
No significant association between VH-IVUS precision rate and stenosis degree was observed in the in vivo arm of the study.

**Conclusion**

The importance of plaque morphology and histology in carotid artery treatment has been largely demonstrated [5-8-18].

The composition assessment of atherosclerotic carotid artery plaque is thereby essential to identify patients at risk of an ischemic cerebrovascular event.

The recent increase of the number of CAS procedures has raised the need for the identification of a diagnostic tool for plaque composition evaluation, which would provide information capable of improving the clinical approach to the disease and the indications for its treatment.

Intravascular ultrasound (IVUS) is an imaging technique that has contributed to our understanding in many areas of interventional cardiology with its capability to assess the extent of vessel remodeling, plaque morphology, and exact vascular dimensions [8-9-13]. Thanks to its safety, simplicity and already widespread use in coronary arteries, this technology has been used also in carotid arteries. Uncertainty on the ability of IVUS to identify plaques susceptible to rupture, fissure, or erosion, as nonculprit plaques in stable patients have often been found to show the same plaque morphological components associated with vulnerable plaque, has been, however, reported. In addition, IVUS resolution, with an axial resolution of 100-200 μm and a lateral resolution of 250 μm, is considered too low to detect thin fibrous caps (50-75 μm), typical feature of vulnerable plaques. These properties, though beneficial for visualizing deep structures, limit imaging of microstructures, like thin fibrous cap, yielding a sensitivity of only 37% for the detection of plaque rupture, resulting in a limitation to the use of the device [19].

In our study, we first performed an *ex vivo* VH-IVUS - Histology correlation in order to validate the ability of VH-IVUS in identifying the different plaque components. We then performed an *in vivo* VH-IVUS -HR-MR correlation to assess VH-IVUS as a valid alternative to HR-MR in the in vivo plaque characterization.

In order to obtain a direct comparison between the techniques, our group attempted at first to perform the ex-vivo evaluation of the same carotid plaque with VH-IVUS, HR-MRI and histology, using the method proposed by Chanetal.[20-21]. However, the imageresolution obtained by our team with this method was not sufficient and, as to avoid significant discrepancies in the interpretation of the plaque composition between radiologists and pathologist, it was decided to compare VH-IVUS and Histology *ex vivo*, and VH-IVUS and HR-MRI in vivo. In relation to the operator-dependence of the IVUS...
method, evaluation of plaque edges was performed by two operators, first independently and then jointly in order to rule out eventual interpretational doubts.

The VH-IVUS - Histology results of our study suggest an excellent correlation for fibrous and fibro-lipidic plaque components, and a good correlation for calcific and necrotic plaque components. Overall concordance between VH-IVUS and Histology in the ex-vivo arm of the study resulted good.

The VH-IVUS - HR-MR results of our study suggest an excellent correlation for fibrous, fibro-lipidic and calcific plaque components, and a good correlation for calcific and necrotic plaque components. Overall concordance between VH-IVUS and HR-MR in the ex-vivo arm of the study resulted good, though VH-IVUS failed to identify fibrous cap fissuration which corresponded to fibrous cap enhancement in 6 images.

Differences between VH-IVUS and histological data were probably attributable to errors introduced by ultrasonography, such as the inappropriate setting of the gain making difficult the differentiation of lipid tissue from fibro-lipid tissue [22-23]. Our results show that VH-IVUS precisely identifies macroscopic calcifications but, in accordance with other studies, diffuse microcalcifications usually remain undetected [24]. Differences between precision rate, sensitivity and sensibility for necrotic and calcific plaque components in our results may be a result of the lower VH-IVUS spatial resolution, of a slight "shadow cone" artifacts determined by large calcifications and by the different prevalence of necrotic and calcific components among the examined plaques.

In our opinion these results suggest a high ability of VH-IVUS in the identification of calcific and necrotic plaque components, but a significantly low ability in the evaluation of their contours. Although this data appear to be related to an underestimation in our study, similar results are provided in the experience of different authors, which interpretate these results as a intraplaque hemorrhage with a not optimal evaluation with VH-IVUS [25].

The main limit of this cross-sectional study is the relatively small number of subjects and the minor number of available histological locations for comparison.

**CONCLUSIONS**

Though a good correlation was observed either in the ex-vivo VH-IVUS-Histology correlation, and in the in-vivo VH-IVUS - HR-MRI comparison, we believe that VH-IVUS is not suitable for the accurate in vivo differentiation between stable and unstable plaques prone to rupture, due to the not optimal assessment of necrotic component, fibrous cap thickness and rupture signs. We believe that VH-IVUS may, instead, be useful when a quick intraprocedural evaluation of a carotid plaque before or after stent placement is required. We, however, believe that these results need further evaluation in larger populations to be confirmed.
References


Personal Information