Atherosclerotic carotid disease, the vulnerable plaque in the vulnerable patient
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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CURRENT IMAGING MODALITIES TO VISUALIZE VULNERABILITY WITHIN THE ATEROSCLEROTIC CAROTID PLAQUE

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*J Vasc Surg 2008;48: 1620-1629*
ABSTRACT

**Background:** There is increasing evidence that plaque vulnerability, rather than the degree of stenosis, is important in predicting the occurrence of subsequent cerebral ischemic events in patients with carotid artery stenosis. The many imaging modalities currently available have different properties with regard to the visualization of the extent of vulnerability in carotid plaque formation.

**Methods:** Original published studies were identified using the MEDLINE database (January 1966 to March 2008). Manual cross-referencing was also performed.

**Results:** There is no single imaging modality that can produce definitive information about the state of vulnerability of an atherosclerotic plaque. Each has its own specific drawbacks, which may be the use of ionizing radiation or nephrotoxic contrast agents, an invasive character, low patient tolerability, or simply the paucity of information obtained on plaque vulnerability. Functional molecular imaging techniques such as positron emission tomography (PET), single photon emission-computed tomography (SPECT) and near infra-red spectroscopy (NIRS) do seem able accurately to visualize and even quantify features of plaque vulnerability and its pathophysiologic processes. Promising new techniques like near infra-red fluorescence imaging are being developed and may be beneficial in this field.

**Conclusion:** There is a promising role for functional molecular imaging modalities like PET, SPECT, or NIRS related to improvement of selection criteria for carotid intervention, especially when combined with CT or MRI to add further anatomical details to molecular information. Further information will be needed to define whether and where this functional molecular imaging will fit into a clinical strategy.
INTRODUCTION

The current indication for intervention in patients with carotid artery stenosis is primarily based on the degree of stenosis and the symptoms.\textsuperscript{1,2} In contrast, it has become apparent that coronary atherosclerosis produces symptoms because of plaque rupture and this risk is determined more by plaque composition than plaque size or degree of stenosis.\textsuperscript{3} The discrepancy in absolute risk reduction after carotid endarterectomy (CEA) in symptomatic and asymptomatic patients highlights the importance of factors other than plaque size and degree of luminal obstruction in determining risk.\textsuperscript{4} Acute plaque rupture with subsequent thrombosis may occur in vulnerable plaques that do not physically appear threatening, whereas other lesions that are more flow-limiting may be dormant and not progress. The vulnerability is largely dictated by plaque morphology, which, in turn, is influenced by pathophysiologic mechanisms at the cellular and molecular level. Additionally, there is a growing notion that plaque instability is important in the etiology of acute cerebral ischemic events in patients with carotid disease.\textsuperscript{5,6} Therefore, in the future patients may be selected for intervention on the basis plaque vulnerability assessed from morphologic characteristics, rather than on the degree of stenosis or the symptoms.

PLAQUE MORPHOLOGY

There are various stages of atherosclerotic plaque development, each with specific histopathologic characteristics. In the mature stage, the plaque has advanced into an occlusive atherosclerotic plaque characterized by smooth muscle cell migration and the formation of a fibrous cap, a lipid-rich necrotic core, and an ever-increasing inflammatory infiltrate. Atherosclerotic plaque rupture associated with inflammation has been correlated with the presence of highly activated macrophages.\textsuperscript{7} Macrophages weaken the extracellular matrix of the fibrous cap through phagocytosis or by secreted proteolytic enzymes, such as cathepsins or matrix metalloproteinases (MMPs), leading to plaque rupture.\textsuperscript{8} This exposes highly pro-thrombotic material, leading to the formation of thrombus, which may result in clinically recognizable events. In this review, we categorize the imaging techniques currently available and discuss specific properties of each technique with regard to visualization and quantification of vulnerability in carotid plaque formation. New developments within this field are also discussed.
CONVENTIONAL IMAGING TECHNIQUES

Angiography

In the North American Symptomatic Carotid Endarterectomy Trial (NASCET) and European Carotid Surgery Trial (ECST) trials, angiography was the gold standard in determining luminal stenosis in carotid extracranial disease.\(^1,2\) On the basis of these two trials stenosis became the most important factor in defining stroke risk. However, additional subset analysis of the NASCET data showed that in 659 patients with stenosis ranging from 70-99%, the risk of stroke increased between 70% and 94% and then actually decreased as occlusion was approached.\(^9\) Other indicators than stenosis alone are thought to be predictive of stroke risk. Already in 1978 it was mentioned by Moore, et al, that the aspect of ulceration as detected on angiography could predict patients at high risk for a subsequent stroke.\(^10\) Although in general, the inter- and intra-observer variability in judging angiographic images is small, the sensitivity and specificity of angiography in detecting ulcerated plaques are less well-defined.\(^6,11\) Many studies compare the angiographic image with the macroscopic appearance of the plaque, but not with histological findings. Therefore, the first 500 patients recruited into NASCET underwent angiography in an attempt to detect ulceration; angiography was subsequently compared to observations during endarterectomy. Sensitivity and specificity of detecting ulcerated plaques using angiography were 46% and 74%, respectively. The positive predictive value of identifying an ulcer was 72%. These results remained unchanged with differing degrees of carotid stenosis and were confirmed by analyses based on receiver operating characteristic methodology.\(^12\) A comparable study design was used for 1671 patients enrolled in ECST; sensitivity and specificity for ulceration were 69% and 47%, respectively.\(^6\) In studies where the radiological appearance was compared to histology of the resected plaque, sensitivity ranged from 44% to 86%, and specificity from 33% to 74%, indicating that results vary extensively.\(^13-15\) Furthermore, the two largest studies comparing angiographic findings to histology showed conflicting results.\(^14,15\) In the first study, a series of 55 resected plaques from both symptomatic and asymptomatic patients was analyzed; no significant correlation was found between angiography and histology (\(P = .410\)).\(^14\) The second study examined 128 resected plaques from symptomatic patients and compared angiographic assessments to histologic features of the plaque, using reproducible semi-quantitative scales. Angiographic ulceration was associated with plaque rupture (\(P = .001\)), intraplaque hemorrhage (\(P = .001\)), large lipid core (\(P = .005\)), less fibrous tissue (\(P = .003\)), and increased general instability (\(P = .001\)). In comparisons between irregular and smooth plaques, a significant but less strong correlation was
found. There are conflicting results for detecting ulceration on angiography. Eliasziw, et al, found that the presence of angiographically defined ulceration is associated with an increased risk of stroke, while Kim, et al, found no significant relationship between angiographic ulceration and neurological symptoms. It may be concluded that ulceration is the only marker that can be detected on angiography, and with a very wide range of sensitivity and specificity. There is little agreement between angiography and histologic observation. The clinical implication of ulceration as a marker has conflicting results. Therefore, plaque morphology assessed by angiography cannot determine accurately which carotid plaque is stable and which is vulnerable.

Ultrasound (US)
Since the 1980s, duplex ultrasonography has been widely used, mainly to measure flow velocity and flow ratios. The severity of stenosis can be determined by combining results of peak systolic velocity (PSV), end-diastolic velocity (EDV) and pre- and poststenotic ratios. In a recent meta-analysis, duplex ultrasonography scan was found to have a sensitivity of 89% and a specificity of 84% in detecting a stenosis degree of 70% to 99% in carotid arteries. The accuracy of detecting a stenosis degree of 50% to 69% was considerably less (sensitivity 36%, specificity 91%).

In addition to flow measurements, echogenicity of different parts of the artery wall and the atherosclerotic plaque can be visualized. Areas with different shades of gray provide information on plaque consistency. Originally, the appearance of a plaque was either classified as echogenic (calcified) or echolucent (non-calcified). Later, in an attempt to decrease observer variability, more detailed classifications were developed, such as the ones proposed by Gray-Weale and Geroulakos. In these studies, grading of plaque morphology was largely based on the ultrasound scan gray scale appearance, which was assessed subjectively by visual inspection of ultrasound scan images. However, these classifications showed a rather weak inter-investigator reliability and little or no agreement with the histologic results. An alternative approach to objectifying the plaque’s structural composition is to quantify its echogenicity by computer-assisted image analysis. There are several possibilities for analyzing images, such as Gray Scale Median (GSM), Pixel Distribution Analysis (PDA), and Virtual Histology (VH).

Gray Scale Median (GSM) values are calculated by digitizing B-mode images and subsequently processing them with Adobe Photoshop (Adobe Systems Inc, San Jose, Calif). Intraluminal blood and the adventitia are chosen as reference
points. The image is normalized by adjusting the gray scale values of the image’s pixels according to the input and output values of the two reference points. The GSM can be used to quantify the echogenicity of the ultrasound scan image and has been found to be a reproducible index of the echogenicity of carotid plaques with low inter-observer and inter-scanner variability (Fig 1).\textsuperscript{23,24}

Whereas GSM measures median brightness of the entire plaque, PDA provides a mapping of individual tissue components in the carotid plaque image. As with GSM, PDA digitizes ultrasound scan images and normalizes the pixel intensities between two constant reference points (blood and arterial adventitia). By comparing maps of pixel intensity with histology, one can determine Gray-Scale Pixel Ranges for different types of tissue. Since pixel intensity correlates with tissue type, one can use the computer to apply a false color scale, creating a form of VH.\textsuperscript{25,26}

Although recent advances in ultrasonographic evaluation of plaques are promising, different ultrasound scan studies have varied widely in both sensitivity and specificity for determining surface ulceration.\textsuperscript{13,18,19,27,28} The same applies to...
the detection of intra-plaque hemorrhage. Moreover, in up to 37% of cases, it is not possible to evaluate the plaque surface due to acoustic shadowing or a high degree of stenosis. With the development of computer-assisted image analysis, inter-observer variability of ultrasound scan analysis has decreased. The Imaging in Carotid Angioplasty and Risk of Stroke (ICAROS) trial showed that increased echolucency of the carotid plaque, defined as a GSM of less than 25, is a risk factor for stroke during and immediately after carotid artery stenting. As the ICAROS trial recruited patients receiving a carotid stent, there was no histologic substrate to compare to the GSM. Denzel, et al, compared B-mode images of 107 carotid endarterectomy specimens and their GSM values to a histologic classification consisting of only three groups (calcium-rich, lipid-rich, and combined plaques). Only 46% of the cases showed agreement between the GSM and the histopathologic findings. Correlation of PDA with histology showed similar results. Currently, it seems that the ability of ultrasound scan to predict signs of vulnerability in the preoperative phase is limited. New computer-assisted image analysis software is being introduced that should improve this technique’s accuracy.

**Intravascular ultrasound (IVUS)**

In addition to being used transcutaneously, the ultrasound scan technique can be applied using miniature transducers small enough to be placed within an angiographic catheter, achieving realtime imaging of vascular structures. With IVUS, the high frequency transducer (20 to 40 MHz) can be placed in proximity to the tissue to be visualized, yielding images of high spatial resolution. This makes it possible to assess the vascular wall from within the lumen and to monitor blood flow as well. Currently, IVUS is used as an integrated monitoring tool in several vascular centers during endograft and stent-graft placement, often to provide additional information on vessel wall morphology in order to achieve optimal stent placement (Fig 2). So far, most of the work done on IVUS comes from the field of coronary heart disease. An IVUS was first described almost 20 years ago in the imaging of coronary arteries. In coronary artery plaques, spectrum analysis of IVUS-derived data has identified four major plaque components, ie, fibrous, fibrofatty, necrotic core, and dense calcified tissue. Recently, Irshad, et al, published their early experiences with VH-IVUS during carotid artery stenting. Five cases were presented in which VH-IVUS was found to give useful plaque mapping. The Carotid Artery Plaque Virtual Histology Evaluation (CAPITAL) study analyzed the use of VH-IVUS in 15 patients who subsequently underwent carotid endarterectomy. The VH-IVUS data were compared to histology of the resected specimens. The diagnostic accuracy varied with the composition of the plaque (from 99% in thin-cap fibroatheroma
A drawback of IVUS is its invasiveness. In the CAPITAL study, 2 out of 15 CEA patients had debris collected from their filters, presumably due to manipulation of the IVUS probe. Further studies will be needed to determine its definitive role during carotid artery stenting (CAS) and other vascular interventions.

Computed tomography (CT)
A CT-angiography (CTA) is a validated tool for determining the degree of stenosis in the internal carotid artery. Single-slice CTA has been shown to have a sensitivity of 79-87% and a specificity of 88-90% (compared to “gold standard” angiography). A more recent study showed a significant correlation between single-slice CTA and angiography regarding degree of carotid stenosis, with 100% sensitivity and 100% specificity using CTA to depict internal carotid artery (ICA) stenosis greater than 70%.

Assessment of arterial wall morphology with CTA is clearly not as reliable as assessment of luminal narrowing. Research has focused on three major topics, i.e., ulceration, calcification, and morphology/plaque composition. With regard to detection of ulceration, somewhat disappointing results have been found: sensitivity ranged between 50-94% and specificity between 74-99%. Two studies compared preoperative CTA images to postoperative histology. In both studies, a single-slice scanner was used, which might explain the poor results. Saba, et al, used a multi-slice CT with much better results. However, they did not use histology as control, but instead examined the macroscopic morphology of the plaque for ulceration.

Figure 2. Virtual histology intravascular ultrasound (VH-IVUS) on a carotid stenosis, showing a calcified narrowing with white calcium and green fibrous plaque (with kind permission of Donald B. Reid, MD, FRCS, Wishaw Hospital, Scotland, UK).
There are conflicting results in the detection of calcification with CT. A comparison between CT images and histology with regard to the amount of calcification is often biased through decalcification in the processing of the specimen for histology.\textsuperscript{42,43} Other explanations for the conflicting results are displacement of calcified foci during slide preparation, the inability to detect early stages of mineralization microscopically, or the varying volume incorporated within the CT sections.\textsuperscript{42} Most studies have shown that CT has a significant ability to detect calcification.\textsuperscript{45-47} Some studies showed a relationship between calcification and symptomatology,\textsuperscript{47} while others did not.\textsuperscript{48}

It seems that calcification is the only histological content that can be determined in carotid plaques using CTA. There have been some small studies illustrating the ability of CT to determine plaque morphology.

In 2008, Wintermark, et al, published their results of high-resolution CT imaging of carotid plaques, using a multidetector-row CT (MDCT) and compared the images with histology of the resected carotid plaques. Their study population contained 8 patients and they found an overall agreement of 72.6\% between CTA and histologic examination. For large calcifications, CTA classified the lesions with a 100\% sensitivity and a 100\% specificity. However, a significant overlap between densities associated with lipid-rich necrotic core, connective tissue, and hemorrhage limited the reliability of individual pixel readings to identify these components. Lipid cores were identified with a sensitivity of 76\% and a specificity of 74\%, wide hemorrhages with a sensitivity of 62\% and a specificity of 99\%, and ulcerations with a sensitivity of 87\% and a specificity of 99\%.\textsuperscript{49}

Hardie, et al, found, using MDCT, that expansive carotid remodeling was significantly greater in patients with cerebral ischemic symptoms than in asymptomatic patients. The extent of expansive remodeling may indicate underlying atherosclerotic plaque vulnerability.\textsuperscript{50} Saba, et al, found a similar correlation between thickness of the arterial wall and symptoms. The drawback to these studies is lack of histologic confirmation of the CT images.\textsuperscript{51} A smaller study (n = 9) used CTA to assess plaque density; histologic findings (lipid/hemorrhage, fibrous tissue) were reflected in CTA findings.\textsuperscript{42} The two largest studies (n = 38 and n = 55) with histological confirmation purported that CTA could not depict plaque morphology (lipid, hemorrhage, fibrous tissue, inflammation).\textsuperscript{43,45} A recent study of 14 patients using a multi-slice scanner showed that CTA is capable of characterizing and quantifying plaque burden, calcifications, and fibrous tissue in atherosclerotic carotid plaque, with results that correlated well with histology. The study also had a good inter-observer reliability. However,
the lipid core could only be adequately quantified in certain subsets of plaques, and hemorrhage and thrombus could not reliably be distinguished from lipid.\textsuperscript{52}

In conclusion, CT is currently not useful for predicting vulnerable plaques because it only visualizes the amount of calcification, nor does it have the potential to provide functional pathophysiologic information.

**Magnetic resonance imaging (MRI)**

An MRI’s ability to detect features of plaque vulnerability can be categorized into intra-plaque hemorrhage, necrotic core, fibrous cap, and calcification. Intra-plaque hemorrhage can be detected with a sensitivity of 82-92\% and a specificity of 74-100\%.\textsuperscript{53-56} In addition, MRI can differentiate between hemorrhages of different ages, but with only moderate agreement between MRI and histology ($\kappa = 0.44-0.66$).\textsuperscript{54} Moody, et al, showed that an MRI technique exploiting the $T_1$-shortening properties of recent thrombus can identify histologically-confirmed carotid thrombus, and, thus, complicated plaque.\textsuperscript{57} This technique has the advantage of a short scan time of little over 4 minutes.

A lipid-rich necrotic core can be detected with MRI with a sensitivity of 84-98\% and a specificity of 65-100\%.\textsuperscript{53,55,56,58,59} More recently, in an ex-vivo experiment, pixels were classified into different plaque components and then compared to histological specimens.\textsuperscript{58} Necrotic core could be detected with a sensitivity of 84\% and a specificity of 75\%. An in-vivo study showed that MRI measurements of the lipid-rich necrotic core did not differ significantly from findings on histologic examination of the resected carotid plaque with a strong correlation between MRI and histologic area measurements ($r = 0.75$; $P < .001$).\textsuperscript{55} Gadolinium-enhanced images showed even better results (correlation $r = 0.87$, $P < .001$).\textsuperscript{59} Gadolinium-based contrast agents are known to distribute into the extra-cellular fluid space and a greater degree of enhancement in the vessel wall may be due to (1) increased wash-in of gadolinium-based contrast agent (increased permeability), (2) increased volume of distribution (increased extracellular volume), (3) decreased washout.\textsuperscript{60}

MR imaging can detect an unstable or disrupted fibrous cap with a sensitivity of 81-100\% and a specificity of 80-90\%.\textsuperscript{56,61,62} An unstable fibrous cap may be defined as (1) interruptions or irregularities in the juxta-luminal area; (2) absence of intimal tissue between the lumen and deeper structures; or (3) focal contour abnormalities of the luminal surface.\textsuperscript{62} In a small study ($n = 9$), 5 subjects underwent carotid endarterectomy and subsequent histologic examination. Gadolinium-enhanced images helped discriminate fibrous cap from lipid core.\textsuperscript{60}
Cai, et al, noted that the degree of enhancement of the fibrous cap varied depending on its composition. A fibrous cap with loose matrix, neo-vasculature and inflammatory cell infiltrates was associated with stronger enhancement compared with fibrous caps predominantly composed of organized, dense collagen.\textsuperscript{59} This is consistent with the belief that greater enhancement is seen with increased permeability and increased volume of distribution within the cap.

Overall, MR imaging can detect plaque calcification with a sensitivity of 76-98\% and a specificity of 86-98\%.\textsuperscript{55,56,58} Most studies routinely decalcified their tissues in the histologic processing, but this can be a source for error. Nevertheless, Saam, et al, found an underestimation by MRI of the calcification as percentage of the vessel wall (5.0\% by MRI, 9.4\% by histology; P < .001).\textsuperscript{55} They suggested that calcification shrinks less than other components during histologic processing. Another possible explanation could be that MRI underestimates areas with hypointense signals because of signal averaging of voxels that only partially contain calcification. The resolution of MRI and the inaccuracy that can arise because of signal averaging is thought to improve in the future with the development of better hardware.\textsuperscript{55}

An MRI has a clear advantage in plaque imaging: it does not use ionizing radiation. An MRI has a moderate to good ability to detect a number of plaque components. Despite this, it is challenging to distinguish different intraplaque tissues from each other. For example, both calcification and chronic hemorrhage produce hypointense signals in four separate contrast weightings. Differentiation can only be made by very meticulous examination of the borders of the hypointense signal to detect specific border irregularities, which are characteristic for both plaque components.\textsuperscript{63} Another drawback of MRI is the high percentage (8-28\%) of failed tests because of poor image quality, which is mainly due to motion artifacts. Finally, the use of gadolinium-based contrast agents was considered harmless, but recent reports describe an incidence of nephrogenic systemic fibrosis in up to 3\% of the patients with renal dysfunction who receive gadolinium-based contrast during MRI.\textsuperscript{64}

In conclusion, the use of MRI to create images of vulnerable plaques is promising. However, scan time, image quality, and the possibility to distinguish between different tissues are all issues that need to be improved. Additionally, it is anticipated that by the development of new MR contrast agents for molecular imaging, like magneto-nanoparticles, the accuracy of MR in detecting the vulnerable plaque will improve.
NEW DEVELOPMENTS

The above-mentioned imaging modalities are used for anatomic imaging; they can identify several morphological features of the vulnerable plaque, but provide little or no information regarding cellular and molecular mechanisms. Cellular and molecular mechanisms can be measured by systemic markers. Inflammation is an important part of atherosclerosis and serum markers such as high sensitivity C-reactive protein (hsCRP) are important independent risk factors for future cardiovascular events. However, these markers resemble the entire cardiovascular burden of a patient and do not give information on specific plaques at risk for rupture. New techniques are currently being developed to obtain images of molecular processes within specific plaques, techniques that try to image the morphological properties of the plaque with new methods also denoted as functional molecular imaging.

Optical coherence tomography (OCT)

An OCT is analogous to IVUS; while IVUS measures sound waves, OCT measures the intensity of reflected infrared light. The major advantage of OCT is the spatial resolution, which is approximately 10 times higher than that of an ultrasound scan. However, the major drawback to this invasive technique is the necessity to temporarily displace the blood volume with saline, because blood significantly attenuates OCT images. Another limitation of this technique is the small penetration depth (1-2 mm), which is not enough to obtain an image of the entire vessel wall. Therefore, clinical use is not anticipated.

Thermography

Atherosclerosis is an inflammatory process. Detection of the heat produced by activated macrophages provides a metabolic functional characterization of the atherosclerotic plaque. Temperature variations can be measured with sensors fitted to catheters. A study using carotid endarterectomy specimens and a needle thermistor demonstrated that atherosclerotic plaques exhibit thermal heterogeneity on their luminal surface. Catheter-based measurements of coronary arteries showed an increased difference in temperature and an increased heterogeneity in temperatures within the plaques in patients with ischemic heart disease compared with healthy subjects. Within the group of patients with ischemic heart disease, the differences were greater when the disease was more severe (stable angina vs unstable angina vs acute myocardial infarction). This suggests that progressive disease alters the metabolic state of the atherosclerotic plaque. However, thermography is an invasive technique and less patient friendly. Its true value in clinical application and improved selection of patients needs to be evaluated.
Positron emission tomography (PET) and single photon emission computed tomography (SPECT)

Nuclear imaging modalities are capable of visualizing metabolic activity and molecular processes. PET has an advantage over SPECT in having a 2-3 times better spatial resolution. The spatial resolution for PET is approximately 5 mm and is only useful in larger arteries. The restricted resolution can be partially counteracted by co-registration of scintigraphic images with CT (SPECT-CT and PET-CT hybrid camera systems) or MRI. If PET and SPECT are to gain a position as a clinical instrument in the search for the vulnerable plaque, specific tracers will be needed to image components which play an important role in the formation and progression of vulnerable plaques. The most widely available tracer for analysis of plaque inflammation is ¹⁸F-Fluorodeoxyglucose (FDG). FDG is a glucose analogue that is taken up by glucose-using cells that are metabolically active, and it is phosphorylated by hexokinase. However, no further intracellular metabolization takes place, which results in an accumulation of the tracer intracellularly. FDG is known to accumulate in macrophage-rich areas of carotid plaques.

Figure 3. Fused PET-CT images of a 50-70% symptomatic stenosis on the left side. Gray scale median of the plaque was 10 indicating the existence of an atheromatous non-calcified plaque with high risk for thromboembolism. No FDG uptake was noted at the level of the asymptomatic stenosis on the contralateral side.
Rudd, et al, demonstrated that atherosclerotic plaque inflammation can be imaged with FDG-PET, and that symptomatic unstable plaques accumulate more FDG than asymptomatic lesions. Other radiolabeled SPECT tracers are available for imaging specific plaque vulnerability items, such as \(^{99m}\text{Tc}\)-labeled oxidized low-density lipoprotein (\(^{99m}\text{Tc}\)-LDL) accumulation and apoptosis (\(^{99m}\text{Tc}\)-annexin-V). Preoperative FDG-PET imaging of 17 patients who underwent a carotid endarterectomy showed a significant correlation between the PET signal from the carotid plaques and the macrophage staining from the corresponding histological sections (\(r = 0.70; P < .0001\)). Assessment of macrophages in the specimens was done by the method previously described by Jander, et al, in which macrophages (CD68) and T cells (CD3) were immunocytochemically stained.

Near infrared spectroscopy (NIRS)

NIRS quantitatively and qualitatively detects the chemical composition of an atherosclerotic plaque. A near infrared spectrometer emits light into a sample and measures the proportion of light that is returned over a wide range of optical wavelengths. It is based on the fact that different substances absorb and scatter NIR light to different degrees at various wavelengths. Ex-vivo analysis of freeze-dried sections of carotid endarterectomy specimen measured at room temperature within 10 minutes of harvesting showed significantly different absorption spectra between stable and vulnerable plaques. A catheter-based system has been developed that demonstrated the ability to safely obtain high-quality near infrared spectra in 6 patients with stable angina. Additional studies are planned to validate the technique’s ability to identify lipid-rich coronary artery plaques and ultimately link chemical characterization with subsequent occurrence of an acute coronary syndrome. No FDA approval for clinical use of molecular imaging probes has yet been gained. Another innovative and promising technique relates to the use of NIR fluorescence imaging (NIRF). This technique operates in the near infrared spectrum for imaging of molecular
processes like atherosclerosis. By using so-called smart activatable probes such as MMPSense (VisEn Medical Inc, Woburn, Mass) activated by MMPs like MMP-2 and MMP-9, these optical contrast agents can be visualized in-vivo in dedicated small animal imaging camera systems or in endarterectomy specimen from patients after ex-vivo incubation with MMPSense (Figs 4 and 5). MMP-2 and MMP-9 are considered important biomarkers in the formation of a vulnerable plaque. In-vivo NIRF studies in patients have to be awaited in order to evaluate this new technique on its merits for an improved selection of patients.

![Intraluminal](image1.png) ![Extraluminal](image2.png)

**Figure 4.** Ex-vivo near-infrared fluorescence imaging of intra- and extraluminal sides of resected specimen after carotid endarterectomy. Specimens were analyzed with a patented highly sensitive cooled charge-coupled device camera connected to a light-tight black chamber. Strongest autofluorescence signals were found at dense lipid core sites, whereas the largest accumulation of MMPSense, a protease activatable fluorescent agent that is activated by matrix metalloproteinases (MMPSense 680, VisEn Medical Inc, Woburn, Mass), seemed to occur at shoulder regions of the plaque.
CONCLUSION

There is not yet any superior modality of atherosclerotic plaque imaging for the selection of patients for CEA. First, there is no single imaging modality that can produce definitive information about the level of vulnerability of an atherosclerotic plaque. Most imaging modalities can produce only partial morphological information. Second, all imaging modalities have their own specific drawbacks; they are invasive, use ionizing radiation, or nephrotoxic contrast agents, have low patient tolerability, or simply provide little information about plaque vulnerability. Established techniques such as US and CT need to be improved, while SPECT, PET, MRI, and the associated specific probes and contrast agents for functional molecular imaging need to be further developed on their clinical applicability. Combined techniques like SPECT-CT, PET-CT, and fused PET-MRI may be promising tools for the near future for non-invasive clinical use. The exact role of NIRS and NIRF in the clinical setting needs to be further explored. From all this, we are optimistic that it will be possible to develop a better selection policy for carotid intervention.

Figure 5. Overlay color photography of human carotid endarterectomy specimen and near-infrared fluorescence imaging using the IVIS Spectrum camera system (FOV 15, binning 16, aquisition 1 second, f/stop 2, spectral unmixing at excitation/emission 680/720, epi-illumination). Images were taken immediately after resection (visible light, left panel; autofluorescence, middle panel) and one hour after incubation with MMPSense 680 (right panel). Data are expressed as radiance (ph/sec/cm2/sr) and depicted as a pseudocolor scale on the right. Visible light pictures were fused with the fluorescent data (with kind permission of Johannes S. de Jong, Department of Surgery, University Medical Center Groningen, Groningen, The Netherlands).
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