Chapter 2
Specialised imaging to identify high-risk plaque

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SUMMARY

• Various imaging modalities such as nuclear molecular imaging, including PET and SPECT, and Bio-optical imaging can be used to identify the high-risk carotid plaque but need to be further validated and developed.

• Of all imaging agents, $^{18}$F-FDG is currently the most validated and clinical potential imaging agent to identify the high-risk plaque. In addition to $^{18}$F-FDG, $^{18}$F-NaF is also a promising imaging agent.

• Imaging agents to visualise and quantify proteolytic enzymes especially matrix metalloproteinases-9, can be of great potential to identify the high-risk plaque but validation of imaging agents in humans is complicated and needs to be further developed.

• There is a clear need for large population-based studies for more accurate plaque assessment as a good selection policy for intervention is important.
INTRODUCTION

The development of an atherosclerotic plaque is a complex and dynamic process involving various pathological events such as endothelial cell dysfunction, inflammation, proteolysis, apoptosis, lipid accumulation, angiogenesis, thrombosis, and calcification. A high-risk plaque is usually characterised by a thin vulnerable fibrous cap. If the fibrous cap degenerates, the plaque ruptures and dispels its thrombogenic lipid core into the vessel lumen, potentially leading to an acute vascular event. Several endothelial, inflammatory, and smooth muscle cells have the ability to excrete proteases such as matrix-metalloproteinases (MMPs) and cathepsin cysteine proteases (CCPs). These proteases degenerate extracellular matrix and collagen inside the fibrous cap resulting in a lesion more prone to rupture.

The identification of plaques in patients who are at high-risk for an acute vascular event potentially allows early preventative interventions. It has become clear that the pathological property of an atherosclerotic plaque, rather than its size or the degree of stenosis, is important to identify a high-risk plaque. Conventional anatomic imaging modalities, such as duplex ultrasound imaging, identify stenotic plaques and allow assessment of the degree of stenosis, but they do not provide any information on its pathological state. To allow better clinical risk stratification and to identify a high-risk plaque, there is a clear need for advanced imaging techniques. With targeted, specialised imaging, molecular pathophysiological processes can be visualised and as a consequence, the number of irreversible ischemic events may be reduced (Figure 1).

Figure 1 | Scheme of inflammation and related pathogenic processes occurring of the high-risk plaque and targeted in vivo imaging. Adapted from Chen et al.
Nuclear imaging: PET and SPECT
Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging allow assessment of several in vivo pathological processes within the atherosclerotic plaque. These nuclear medicine techniques are based on the use of radioactive imaging agents. PET has the advantage over SPECT by allowing a more precise quantification of signals as well as localisation of the plaque activity due to a two-to-three times better spatial resolution. Since PET and SPECT imaging are limited in spatial resolution, co-registration with computed tomography (CT) scanning or magnetic resonance imaging (MRI) is necessary for accurate anatomic localisation of the radioactive signal. CT imaging is very effective for detailed vascular imaging because of its high spatial resolution, accompanied by a short acquisition time. Combining cameras such as hybrid PET/CT is a reliable method to visualise and quantify atherosclerosis and inflammation. However, co-registration with MRI has some additional advantages. MRI is superior to CT in that it provides better soft tissue contrast and a precise analysis of the arterial wall without exposure to radiation.

Bio-optical imaging
Bio-optical imaging is a technique based on visible, ultraviolet, and infrared light to obtain molecular imaging without the need of radiation. An additional advantage of bio-optical imaging is visualising and measuring different properties of tissue at the same time due to use of various wavelengths of light. In the field of bio-optical imaging, bioluminescence and fluorescence are the most commonly used techniques. Use of bio-optical imaging is hampered by limitations such as the short penetration depth of the fluorescent signal, high costs, and the complexity of the tracers and camera equipment. However, despite all disadvantages, intra-operative use of these techniques is currently under development in oncology, and we expect clinical cardiovascular application of optical imaging agents to follow in the near future.

Bioluminescence
Bioluminescence imaging is based on the capacity of several organisms to produce light by an enzyme-catalysed reaction. The pigment luciferin is administered and oxidised by an enzyme called luciferase, resulting in the emission of light without the use of an external light source. This process can be used to non-invasively visualise biological processes. However, until now, bioluminescence imaging was restricted to experimental approaches, as cells or whole organisms always need to be transfected with the luciferase gene before luciferase can be expressed.
**Fluorescence imaging**

In fluorescence imaging, an external light of a certain wavelength is used to excite a fluorescent molecule. The excited molecule will almost immediately release a longer wavelength, lower-energy light to enable imaging. Fluorescence, especially the use of light in the near-infrared fluorescence (NIRF) spectrum, contributes to a highly versatile platform for *in vivo* molecular imaging. The sensitivity for detection of certain processes with NIRF imaging exceeds that which can be detected with other molecular imaging modalities. Pathological processes that can be measured include endothelial cell dysfunction, inflammation, proteolysis, apoptosis, and thrombosis. For direct fluorescence imaging of these processes probes targeting a specific receptor or an enzyme are necessary. The use of fluorescent imaging probes to identify the high-risk plaque is a promising modality, but clinical proof-of-concept studies are necessary.

![Image](image.png)

**Figure 2** | Imaging results from intact plaques made with MSOT. The colour images were taken in a cryo slicer system. The fluorescent images were taken from 50 micron cryo section. MSOT morphologic reconstruction and the reconstruction from the MMPSense 680 signal. Obtained from experiments performed at our department.
Multispectral optoacoustic tomography
The problem with NIRF is that penetration of light is limited by tissue scattering. This scattering degrades the spatial resolution and overall accuracy, especially at increased penetration depths. Besides, the previous Bio-optical imaging techniques result in two-dimensional images. Using multispectral optoacoustic tomography (MSOT), it is possible to generate a three-dimensional image (Figure 2). Optoacoustic imaging is based on the generation of the optoacoustic effect, in which pulses of laser-light that are absorbed in tissue giving rise to hyperthermia followed by broadband ultrasound waves, which can be easily non-invasively detected.

Imaging agents for the high-risk plaque
In molecular imaging, imaging agents are labelled with radioactivity or fluorescence (or other suitable dyes) to visualise different pathological processes.

Figure 3 | Examples of in vivo imaging of a symptomatic carotid plaque. Clinical PET/CT image with coronal plane slice of a patient showing ¹⁸F-FDG uptake in the affected right carotid artery (A). Obtained from previously published research. Clinical SPECT/CT image with coronal plane slice of a patient showing IL-2 uptake at the location of the near-occlusion symptomatic plaque in the affected right carotid artery (B).
Inflammation

Inflammation of the arterial wall plays a key role in the development of a high-risk atherosclerotic plaque. The most commonly used imaging agent is the radioactively labelled glucose molecule $^{18}$F-2-fluoro-2-deoxy-D-glucose ($^{18}$F-FDG) (Figure 3). $^{18}$F-FDG is especially consumed by cells with a high metabolic rate. The $^{18}$F-FDG signal has been shown to be significantly associated with macrophage infiltration and levels of inflammatory activity in carotid plaques in ex vivo studies. Previous in vivo studies have demonstrated that the vascular $^{18}$F-FDG signal was associated with inflammatory biomarkers, early recurrent stroke, and even predicted cardiovascular events independent of traditional risk factors in asymptomatic adults. In clinical trials, $^{18}$F-FDG uptake has also been used successfully as a monitoring tool for evaluating anti-atherosclerotic therapies.

Another PET tracer that has been studied in humans for evaluating atherosclerotic plaques and inflammation is $^{68}$Ga-DOTATATE. This tracer binds to somatostatin receptor 2 which is expressed on activated macrophages. Previous studies have shown that the vascular $^{68}$Ga-DOTATATE uptake correlated with cardiovascular risk factors.

In addition to macrophages, lymphocytes play a significant role in development of a high-risk plaque. If lymphocytes are activated, they stimulate macrophages to produce MMPs. IL-2 is a pro-inflammatory cytokine, which is produced by T lymphocytes and associated with an increased carotid artery intima media thickness (a predictor of stroke). The IL-2 receptor is over expressed on activated T lymphocytes during inflammation. IL-2 can be radiolabelled as its regular drug derivative, aldesleukin. However, the labelling procedure is complex and long, mainly due to aldesleukin instability during the labelling procedure. Several groups have demonstrated that $^{99m}$Tc-IL-2 accumulated in symptomatic carotid plaques and correlated with the amount of IL-2R+ cells, and T lymphocytes within the plaque.

Proteolysis

An important process in plaque progression is the metabolic activation of the fibrous cap. Metabolic activation will be triggered from the release of proteolytic enzymes such as MMPs and CCPs. For example, $^{99m}$Tc-labeled MMP inhibitors showed a higher uptake in carotid artery stenosis compared with normal arteries in mice. Furthermore, in another ex vivo study in which MMP-9 was visualised with NIRF imaging, MMP-9 was also shown to have an important role in the pathogenesis of plaque rupture. Nevertheless, the relation between MMP expression and stroke needs to be further established and imaging agents should be validated in humans instead of animals.
Apoptosis
Carotid plaques with an increased necrotic core due to extensive apoptosis of macrophages appear to be closely associated with higher likelihood of plaque rupture.\textsuperscript{17} Apoptotic cells start to express phosphatidylserine on the outside of the membrane. Annexin-A5 has a high affinity for phosphatidylserine and can be labelled with either $^{99m}$Tc or $^{18}$F to serve as an imaging agent. In a proof of concept study of four patients with a history of a transient ischaemic attack as a result of carotid artery stenosis, \textit{in vivo} Annexin-A5 uptake corresponded with histopathological analysis of the high-risk plaque.\textsuperscript{18} Unstable plaques showed higher uptake of Annexin-A5. There are also other imaging agents that bind to phosphatidylserine to detect apoptosis. Synaptotagmin C2A is a peptide, which has been conjugated to magnetic nanoparticles for MRI as well as $^{99m}$Tc for nuclear imaging.\textsuperscript{19} However, more research is needed to validate this peptide in humans.

Lipid accumulation
The extent of the lipid core is critical to the stability of the high-risk plaque. High-risk plaques were shown to have a much larger central lipid pool.\textsuperscript{20} There are several imaging agents available to image lipid accumulation such as $^{99m}$Tc-LDL, $^{99m}$Tc-oxLDL, and $^{99m}$Tc-LOX-1, but most of those agents are evaluated in dated studies. However, high lipid accumulation can also be measured by MRI.\textsuperscript{21}

Angiogenesis
Intraplaque angiogenesis is associated with plaque destabilisation. Neoangiogenesis causes plaque growth and is a source of intraplaque haemorrhage.\textsuperscript{22} The intraplaque release of several angiogenic cytokines, such as vascular endothelium growth factor (VEGF), and the local hypoxic environment stimulates angiogenesis. As such, VEGF is a target for imaging. For example, $^{89}$Zr-bevacizumab PET for targeting of VEGF-A has been shown to correlate with immunohistochemistry scores related to plaque instability in human carotid plaque in an \textit{ex vivo} study.\textsuperscript{23} Although it has been suggested that VEGF may have a protective role in atherosclerosis due to regeneration of endothelium, the overall evidence underlines a substantial role in plaque rupture, due to the formation of immature capillary vessels. To explain this discrepancy, further evaluation is needed. However, the use of radioactive $^{89}$Zr-bevacizumab in a clinical setting has a high radiation burden. The latter can be drastically reduced by using $^{18}$F-labelled, labelled to smaller VEGF proteins, such as fab-fragments.\textsuperscript{23}

Calcification
Microcalcification is another feature of high-risk plaques that develops in response to inflammation. While macrocalcification is considered a characteristic of plaque stability, microcalcifications may be related to plaque rupture. Microcalcifications are associated with plaque inflammation and necrosis.\textsuperscript{24} Detection of microcalcification is not possible with a
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CT scan since it only identifies macrocalcification (Figure 4). The feasibility of $^{18}$F-sodium fluoride ($^{18}$F-NaF) PET to visualize microcalcification in the atherosclerotic plaque was recently demonstrated. Currently, $^{18}$F-NaF is the only available clinical imaging agent that can non-invasively detect microcalcification in vascular plaque activity. Additional clinical trials are required to evaluate the value of $^{18}$F-NaF PET for the prediction of cardiovascular events.

Figure 4 | Transverse view of a heavily calcified CEA specimen. Photograph of cut segment after scanning procedure (A). $\mu$PET image of CEA specimen incubated with $^{18}$F-NaF; blue corresponds with low uptake and red is correlated with high uptake (B). CT-image of the same CEA specimen (C). Fused $\mu$PET and CT image (D). Obtained from previously unpublished experiments performed at our department.

CONCLUSION

Specialised molecular imaging techniques to identify a high-risk plaque are available but further evaluation is required to validate imaging agents. Clinical studies are needed to establish the predictive value of these imaging agents and to evaluate their applicability as a surrogate endpoint in clinical trials. Until now, only $^{18}$F-FDG is more or less clinically established to be used as a radiopharmaceutical for imaging of inflammation in atherosclerosis.

Hybrid imaging systems such as PET/CT and PET/MRI can play a pivotal role in this, including the use of whole body vascular imaging. Most promising tracers are $^{18}$F-FDG and $^{18}$F-NaF for hybrid imaging in the near future. Bio-optical imaging without using potentially harmful radiation is a technique with clinical potential but needs to be further developed and validated in humans.
REFERENCES


