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Abstract

A major contributor to the occurrence of ischemic stroke is the existence of carotid atherosclerosis. A vulnerable carotid atherosclerotic plaque may rupture or erode, thus causing a thrombotic event. Currently, clinical decision-making with regard to carotid endarterectomy or stenting is still primarily based on the extent of luminal stenosis, estimated with CT angiography and/or (duplex) ultrasonography. However, there is growing evidence that the anatomic impact of stenosis alone has limited value in predicting the exact consequences of plaque vulnerability. Various molecular processes have, independently of degree of stenosis, shown to be importantly associated with the plaque's capability to cause thrombotic events. These molecular processes can be visualized with nuclear medicine techniques allowing the identification of vulnerable patients by non-invasive in vivo SPECT(/CT) and PET(/CT) imaging. This chapter provides an overview of SPECT(/CT) and PET(/CT) imaging with specific radiotracers that have been evaluated for the detection of plaques together with a future perspective in this field of imaging.

21.1 Introduction

Cerebral infarction and transient ischemic attacks (TIAs) are frequently caused by embolism from an atherothrombotic plaque or thrombosis at the site of plaque rupture (Bamford et al. 1991). Although the degree of anatomic lumen obstruction is a relevant marker of the risk of stroke (Halliday et al. 2004), the recognition of the role of the vulnerable plaque has opened new avenues in the field of atherothrombotic stroke. Vulnerability is caused in part by the plaques' tissue structure, which, in turn, is influenced by pathophysiologic mechanisms at the cellular and molecular level. All types of atherosclerotic plaques with a high chance on thrombotic complications and rapid progression should be considered as vulnerable plaques causing part of clinical events (Naghavi et al. 2006). However, not all (vulnerable) plaques become symptomatic and will result in a stroke or a TIA. Removal of carotid artery stenosis by surgical endarterectomy or treatment with stenting will reduce stroke incidence markedly. On the other hand, approximately 3–9% of patients undergoing such an interventional treatment will suffer from stroke or even death as a complication from it. Stenting procedures are generating a higher risk for major complications than carotid endarterectomy (van der Vaart et al. 2008). Current selection criteria for intervention are determined by the grade of stenosis and symptomatology. It is generally accepted to be more aggressive in a high-grade symptomatic carotid stenosis. Invasive interventions in lower-grade stenosis/low frequency of symptomatology are still a matter of debate. Currently conventional available imaging modalities, such as ultrasound, angiography, CT angiography, or MR angiography, can delineate vascular wall anatomy and the severity of stenosis. However, evidence is growing that stenosis severity

alone has limited value in predicting plaque vulnerability and its associated risk for cerebral ischemic events (Sanidas et al. 2012). Furthermore, various pathological processes have, independent of the degree of stenosis, shown to be importantly associated with plaque vulnerability. Imaging the target biologic processes is important in the evaluation of plaque evolution and plaque (in)stability. Expansion of the vessel wall involving remodeling of the extracellular matrix can be imaged, as can angiogenesis of the vasa vasorum, plaque inflammation, proteolysis, lipid accumulation, apoptosis, and fibrin deposits in early non-occlusive atherosclerosis. Several imaging platforms are available for targeted vascular imaging to acquire information on both anatomy and pathobiology. This can be achieved in the same imaging session using hybrid technology with novel tracers targeting processes identified by molecular biology to be of importance. In this chapter we focus on visualizing molecular processes with nuclear medicine/molecular imaging techniques allowing the identification of vulnerable plaques by non-invasive in vivo SPECT(/CT) and PET(/CT) imaging.

21.2 Background of Plaque Vulnerability

Atherosclerotic lesions arise from focal thickenings of the intima of arteries of any size. It is initiated by a process of inflammatory damage, existing of lipid accumulation, inflammatory and smooth muscle cells, as well as connective tissue systematically deposited in these lesions (Fig. 21.1). Low-density lipoprotein (LDL) particles in excess infiltrate the artery wall and are retained in the intima, predominantly at sites of hemodynamic stress flow (Skalen et al. 2002). Local oxidative and enzymatic modifications of LDL particles lead to the release of inflammatory

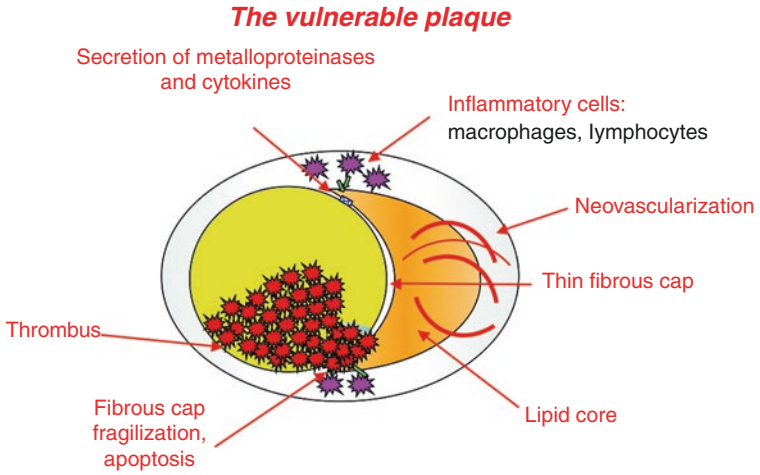

Fig. 21.1 Biological factors supporting plaque vulnerability (Nighoghossian et al. 2005). (Permission to use figure and legend granted by Lippincott, Williams & Wilkins; http://lww.com)

lipids that induce endothelial cells to express leukocyte adhesion molecules such as vascular cell adhesion molecule like (VCAM)-1, E-selectin, and P-selectin. These in turn facilitate the recruitment as well as the subsequent attachment to the artery wall of various white blood cell types, such as T lymphocytes and monocytes (Falk 2006; Hansson 2005). Monocytes differentiate into macrophages, a process described to be associated with the upregulation of pattern recognition receptors including scavenger receptors and Toll-like receptors. These processes lead to foam cell formation. In turn, these release growth factors, cytokines, metalloproteinases, and reactive oxygen species which have their particular influence on the vascular remodeling process. During the atherosclerotic process, endothelial cells, macrophages, and smooth muscle cells die from apoptosis or necrosis (Littlewood and Bennett 2003). Disintegration of foam cells, loss of smooth muscle cells, and production of matrix metalloproteinases by activated leukocytes have detrimental consequences. These processes will finally lead to the formation of destabilizing lipid-rich cores and fragile and rupture-prone fibrous caps (Falk 2006; Jones et al. 2003). The occurrence of VEGF is also linked to advanced atherosclerosis and is probably a marker of ongoing pathologic activity, resulting in high-risk plaques. Also, the progression of atherosclerotic plaques is associated with the appearance and growth of vasa vasorum (Hansson 2005; Barger et al. 1984). In human plaques, microvessel content increases with plaque progression and is likely to be stimulated by plaque hypoxia, hypoxia-inducible factor (HIF) signalling, reactive oxygen species, and other inflammatory signals (Sluimer and Daemen 2009). The presence of plaque hypoxia is primarily determined by plaque inflammation (increasing oxygen demand), while the contribution of plaque thickness (reducing oxygen supply) seems to be a minor factor. Plaque microvessels are immature and fragile, and the distorted integrity of microvessel endothelium likely leads to intraplaque hemorrhage and plaques at increased risk for rupture (Sluimer et al. 2009).

These various stages of atherosclerotic plaque development, with specific histopathologic characteristics, may result in clinically recognizable events.

Further in this chapter, we will categorize the nuclear imaging techniques using different radiopharmaceuticals that are currently available to visualize these various stages. We will discuss their specific properties with regard to visualization of the different pathways associated with vulnerability, including inflammation, proteolysis, apoptosis, angiogenesis, and lipid accumulation in carotid plaque formation.

21.3 Functional Imaging of Carotid Artery Plaque with SPECT/CT and PET/CT

Nuclear imaging modalities are capable of visualizing molecular processes. Clinical positron emission tomography (PET) has a 2–3 times better spatial resolution than single-photon emission computed tomography (SPECT) and does not require the presence of a collimator.

The spatial resolution for PET is approximately 5 mm and is only useful in large arteries. Also PET has shown to be capable of quantifying tracer activity in the region of interest. Restricted resolution can be partially counteracted by coregistration of scintigraphic images with CT (SPECT/CT and PET/CT hybrid camera systems) or with MRI. CT and MRI will be helpful for partial volume correction, but most importantly the advantage of coregistration is precise anatomic information concerning tracer uptake and to visualize aspects that are not depicted by PET and SPECT, such as stenosis and the plaque extent. 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 (Table 21.1) (Glaudemans et al. 2010).

Table 21.1 Radiopharmaceuticals for imaging atherosclerosis

Radiopharmaceutical	Target
	Lipid accumulation
^{99m}Tc-LDL/oxLDL/ac-LDL	Foam cells
^{99m}Tc-LOX-1-mAb	Foam cells
^{99m}Tc-β-VLDL	Lipoproteins
^{125}I/^{99m}Tc-MDA2, ^{125}I-IK17	Lipids
	Inflammation
^{99m}Tc/^{125}I-MCP-1	Macrophages and monocytes
^{68}Ga-SST-2 receptor	Macrophages
^{99m}Tc/^{123}I-IL-8	Neutrophils
^{123}I-IL-1 RA	Monocytes and lymphocytes
^{123}I- or ^{99m}Tc-IL-2	Lymphocytes
^{99m}Tc-Fucoidan	P-selectin
^{18}F-FDG	Glucose activity
	Calcification
^{18}F-Sodium fluoride	Active mineral deposition
	Thrombosis
^{111}In-Platelets	Platelets
^{99m}Tc-Apcitide/P280	Activated platelets
^{99m}Tc-DMP444	Activated platelets
^{99m}Tc-Fibrin-binding domain (FBD)	Fibrins
^{99m}Tc-labelled fibrin α-chain peptide	Fibrins
	Apoptosis
^{99m}Tc-Annexin A5	Apoptotic cells
	Angiogenesis
^{111}In-Bevacizumab	VEGF activity
^{123}I-VEGF$_{165}$	VEGF receptor
^{99m}Tc-Endothelin	Smooth muscle cells
^{11}C-L-159,884	Angiotensin 1 receptor
^{89}Zr-Bevacizumab	VEGF activity
^{18}F-Galacto-RGD	αvβ3 integrin receptor
^{99m}Tc-MMP-tracers	Metalloproteinase (several subtypes)

21.3.1 Inflammation

Inflammation plays a key role in early atherosclerotic plaque development and in plaque destabilization. Especially, macrophages play a central role in plaque pathogenesis. By ingesting lipids, they transform into foam cells and produce a large array of pro- and anti-inflammatory cytokines. The highly inflammatory vulnerable plaque is characterized by an abundance of inflammatory cells and proteins that are all potential targets for molecular imaging tracers. Therefore, molecular imaging of inflammatory activity in the plaque may predict vulnerability and stroke risk. The most widely studied imaging method to identify inflammatory processes in carotid artery plaques is radionuclide scintigraphic imaging by PET. Radionuclide tracers for macrophage metabolism, macrophage recruitment, and inflammatory markers have also been developed and have potential to image inflammatory activity (Masteling et al. 2011). ^{18}F-Fluorodeoxyglucose (FDG) is a glucose analogue that is taken up by glucose-using cells and accumulates in proportion to metabolic activity. FDG-PET has extensively been used in cancer patients. Some cancer patients undergoing FDG-PET showed uptake of FDG in the large arteries, and they were retrospectively identified as having risk factors for atherosclerosis (Yun et al. 2002). This suggested that atherosclerotic plaques may be detectable by FDG-PET imaging. In atherosclerosis, FDG-PET is thought to identify only those plaques that are most actively inflamed and at highest risk for instability and is therefore a potential method for non-invasive identification of an unstable carotid plaque (Fig. 21.2). An early clinical study using FDG-PET in carotid artery plaques was performed in 2002, showing more accumulation of FDG in unstable plaques (Rudd et al. 2002). FDG-PET signals have also shown to correlate with histological macrophage staining or macrophage markers in human atherosclerotic plaques (Masteling et al. 2011). In another clinical study, 12 patients with a recent TIA underwent FDG-PET and high-resolution MRI. FDG uptake was high in 83% of patients with lesions that were compatible with the patients' presenting symptoms (Davies et al. 2005). In

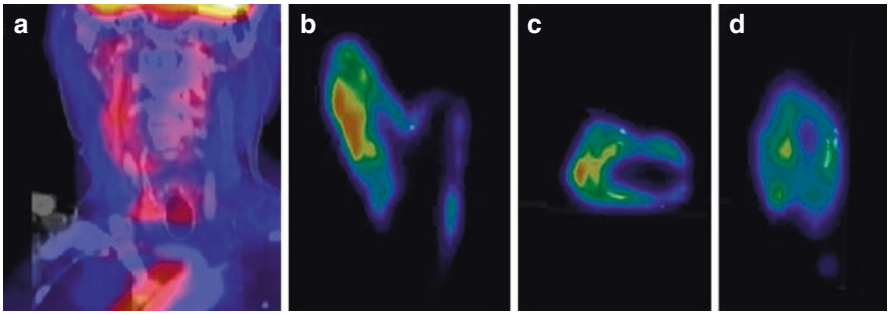

Fig. 21.2 Clinical fused PET-CT image from a patient showing FDG uptake in the affected right carotid artery (**a**). Coronal (**b**), transverse (**c**), and sagittal plane (**d**) of corresponding μPET images in the same patient showing also patchy FDG uptake and calcified areas (white depositions) (Masteling et al. 2011)

another study, concerning new symptomatic carotid stenosis, inflammation-related FDG uptake was associated with early stroke recurrence, independent of the degree of stenosis. FDG-PET may identify patients at highest risk for stroke recurrence, who may be selected for immediate revascularization or intensive medical treatment (Marnane et al. 2012). Bucerius et al. showed that carotid inflammation as revealed by FDG-PET is also highly prevalent in the coronary artery disease population and is associated with obesity, age over 65 years, history of hypertension, smoking, and male gender. Artery wall FDG uptake increased when components of the metabolic syndrome clustered (Bucerius et al. 2011). In a large cohort of 932 cancer patients, increased FDG uptake in major arteries emerged as the strongest predictor of a subsequent vascular event. Concomitant severe vascular calcifications seemed to impart a particularly high risk (Rominger et al. 2009). FDG uptake within an arterial wall or plaque can be quantified in several ways, but there is always a partial volume error (PVE), depending on the spatial resolution of the imaging technique that is being used (Rousset et al. 1998), even though PET has a 2–3 times better spatial resolution than SPECT. Izquierdo-Garcia et al. studied the reproducibility of methods of quantification by MR-guided FDG-PET in symptomatic carotid artery plaques and compared quantification methods to a gold standard technique using the Patlak analysis (Izquierdo-Garcia et al. 2009; Patlak et al. 1983). MR-guided FDG-PET proved to be a highly reproducible technique. A recent meta-analysis includes 14 articles where comparing FDG-PET uptake in symptomatic versus asymptomatic disease yielded a standard mean difference of 94 (95% CI 0.58–1.130; $P < 0.0001$, $I^2 = 65\%$) (Chowdhury et al. 2018). A major disadvantage of FDG imaging is its non-specificity. A FDG-positive scan of the carotid area may indicate plaque formation but may also reflect physiological tracer uptake in muscle or brown fat. CT and MRI may be helpful to distinguish vascular from extravascular uptake. However, there is still a need for more specific PET tracers which are suitable for the detection of the unstable carotid plaque.

While ample data exist about the pattern of FDG uptake in the arterial tree and its colocalization with plaque calcification, only limited data exist for choline and fluorocholine. ^{18}F-labelled fluorocholine (^{18}F-fluorocholine) has been introduced for imaging of the brain and diagnosis of prostate cancer. Choline is taken up into cells by specific transport mechanisms and is phosphorylated by choline kinase. Afterward, it is metabolized to phosphatidylcholine and eventually incorporated into cell and organelle membranes. Increased choline uptake has been shown in tumor cells and activated macrophages.

Bucerius et al. reported the first data correlating ^{18}F-fluorocholine uptake and morphological wall alterations in aortic and common iliac arteries of five patients (Bucerius et al. 2008).

Their observations suggested that fluorocholine uptake and calcification sites are mostly not colocated, similar to the findings of previous studies with FDG (Fig. 21.3).

Another study found out that ^{11}C-choline uptake and calcification in the aortic and common carotid arterial walls are common in elderly men (Kato et al. 2009). Radiotracer uptake and calcification are, however, only rarely colocalized.

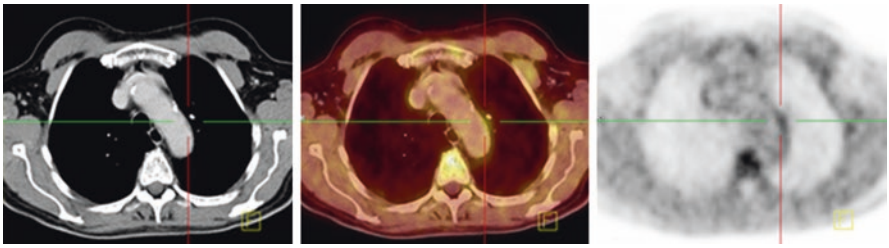

Fig. 21.3 Example of arterial ^{18}F-choline uptake and calcifications representative transaxial ^{18}F-choline-PET/CT results: partially calcified atherosclerotic plaque within the aortic arch which accumulates ^{18}F-choline mainly in the non-calcified part in a 62-year-old (Forster et al. 2010). (**a**) (From left to right) CT image; (**b**) PET/CT fusion; (**c**) PET image

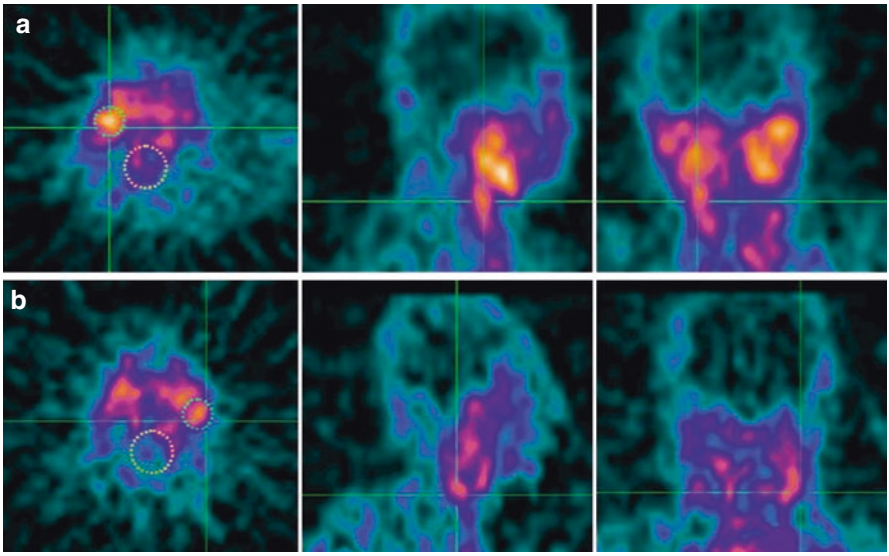

Fig. 21.4 ^{99m}Tc-IL2 scintigraphy in two patients with a plaque in the right (**a**) and the left (**b**) carotid. Images are transaxial, sagittal, and coronal views at the level of the carotid bifurcation. Circles are drawn on the carotid region (small), and larger circles are drawn on the vertebral regions, serving as background to calculate the uptake ratio (carotid/background) (Annovazzi et al. 2006)

^{11}C-choline has the potential to provide information about atherosclerotic plaques independent of calcification measurement with CT.

Another radionuclide tracer that has been used in carotid artery patients is ^{99m}technetium-interleukin-2 (^{99m}Tc-IL-2) SPECT. Higher serum IL-2 levels are associated with increased carotid artery intima-media thickness (IMT), a predictor of stroke and vascular disease (Elkind et al. 2005). ^{99m}Tc-IL-2 is therefore also used for imaging carotid atherosclerosis in humans (Fig. 21.4). Fourteen patients (16 plaques) eligible for endarterectomy underwent ^{99m}Tc-IL-2 scintigraphy before surgery. Another 9 patients (13 plaques) received atorvastatin or a standard

hypocholesterolemic diet, and scintigraphy was performed before and after 3 months of treatment. ^{99m}Tc-IL-2 accumulated in vulnerable carotid plaques, and the accumulation correlated with the amount of IL-2R+ cells within the plaque (measured ex vivo by histology). Also, the amount of ^{99m}Tc-IL-2 within the plaque was influenced by lipid-lowering treatment with a statin (Annovazzi et al. 2006). ^{99m}Tc-IL-2 seems a promising tracer that could provide useful information for the selection of infiltrated vulnerable plaques at risk of rupture. However, it is not yet commercially available, and a major drawback is the complexity and duration of the labelling procedure (Signore et al. 2003). Furthermore, development of the PET tracer ^{18}F-IL-2 for clinical use could be of additional importance since it offers higher resolution (Di Gialleonardo et al. 2012). A new agent for more specific macrophage imaging is the somatostatin receptor subtype-2 (SST2) binding PET tracer. ^{68}Ga-DOTATATE ligand binding to SST2 receptors occurred in CD68-positive macrophage-rich carotid plaque regions. Tarkin et al. validated ^{68}Ga-DOTATATE PET as a novel marker of atherosclerotic inflammation and confirmed that ^{68}Ga-DOTATATE offers superior coronary imaging, excellent macrophage specificity, and better power to discriminate high-risk versus low-risk coronary lesions than FDG (Tarkin et al. 2017). In conclusion, of all radionuclide tracers, ^{18}FDG has currently the most validated and greatest clinical potential to identify inflamed plaques in patients with carotid artery disease, although target binding is not very specific.

21.3.2 Lipid Accumulation

Lipid accumulation not only plays a role in the initial phases of atherogenesis but also is important in the stability of the atherosclerotic plaque. Unstable lesions were shown to have a much greater area occupied by lipids (Dangas et al. 1998). In addition, elevated oxidized low-density lipoprotein (LDL) levels play a role in the transition from stable to unstable plaques and are associated with a higher risk for atherosclerotic complications. In carotid artery disease, technetium-99m labelled LDL (^{99m}Tc-LDL) was used for identification of plaque and appeared to accumulate depending on plaque composition (Lees et al. 1988). Another study in carotid artery plaques compared ^{99m}Tc-oxLDL uptake between normal carotid arteries and patients with a carotid artery stenosis. They found significantly higher uptake of ^{99m}Tc-oxLDL in carotid plaques compared with normal carotid arteries. However, the relationship between ^{99m}Tc-oxLDL uptake and plaque vulnerability by comparison between stable and unstable plaques was not assessed (Iuliano et al. 1996). Although the clinical experience in imaging lipid accumulation is limited, some results suggest that at-risk plaques with high lipid content may be detected by non-invasive imaging in the future.

21.3.3 Proteolysis

Release of proteolytic enzymes such as matrix metalloproteinases (MMPs) and cathepsin cysteine proteases (CCPs) has been suggested as a mechanism of cap

erosion and thus plaque destabilization. Several studies in atherosclerotic plaques have shown proteolytic activity in relation to plaque instability (Loftus et al. 2000; Morgan et al. 2004). Therefore, non-invasive visualization and quantification of MMP and CCP activity is of great potential in risk assessment of carotid artery pathology. Radiolabelled molecules designed to specifically target proteolytic activity have been developed for SPECT and PET. For example, radiolabelled MMP inhibitors that bind to a broad spectrum of MMPs have been studied in mice and showed higher uptake in carotid artery stenosis compared to normal arteries (Schafers et al. 2004). Histological analysis showed colocalization of the specific tracer and MMP-9. In rabbits, MMP activity could be detected by non-invasive SPECT imaging and correlated well with immunohistochemically verified macrophage infiltration and presence of MMP-2 and MMP-9 within the atherosclerotic plaque. In addition, statin therapy and dietary modification were shown to decrease MMP activity as visualized by SPECT, and again immunohistochemical correlations were high (Fujimoto et al. 2008). This indicates that SPECT may be an important tool for monitoring the effects of new therapeutic strategies. However, most of the tracers have only been validated in animal studies, and only ^{111}In-DTPA-N-TIMP-2 has been tested in humans (Van de Wiele and Oltenfreiter 2006). The development of new tracers for atherosclerotic plaques is complicated by a poor target-to-background ratio, which means that background activity makes it hard to distinguish plaque uptake from surrounding healthy tissue.

21.3.4 Apoptosis

Apoptosis also plays an important role in human atherosclerotic plaque progression. The risk of plaque rupture depends in part upon the presence of a necrotic core, containing lipids, dead cells, and debris with a thin fibrous cap (Libby and Theroux 2005). Carotid artery plaques with an increased necrotic core and a thin fibrous cap due to apoptosis of macrophages or smooth muscle cells are known to be instable and are at high risk for rupture.

Imaging of apoptosis in carotid artery plaques has mainly been performed in animal models by targeting markers of apoptosis such as annexin A5. In a rabbit model, Technetium-99m-labelled annexin A5 showed a higher uptake in atherosclerotic lesions compared with controls (Kolodgie et al. 2003). In another rabbit study, annexin A5 (^{111}In-labelled annexin A5) was imaged in combination with MMP activity (^{99m}Tc-labelled matrix metalloproteinase inhibitor (MPI)) using SPECT/CT (Haider et al. 2009). Annexin A5 and MPI uptake were both visualized in atherosclerotic animals and were interrelated.

In a clinical pilot study of four patients with a history of TIA caused by symptomatic carotid artery stenosis, annexin A5 uptake corresponded well with histopathological characterization of vulnerability of the endarterectomy specimens (Fig. 21.5) (Kietselaer et al. 2004). Unstable plaques showed higher uptake of annexin A5, while in stable plaques no uptake of annexin A5 was seen after SPECT imaging. Although obviously more clinical studies in apoptotic markers are needed,

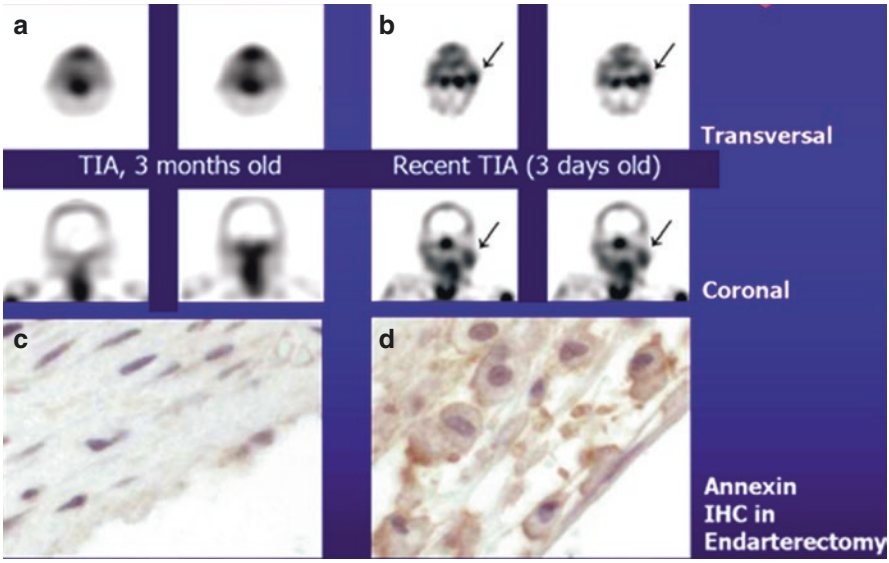

Fig. 21.5 Panel **a** shows transverse and coronal views obtained by single-photon emission computed tomography (SPECT) in a patient who had had a right-sided TIA 3 months before imaging, but did not show evidence of annexin A5 uptake in the carotid-artery region on either side. Doppler ultrasonography revealed a clinically significant obstructive lesion on the affected side. Histopathological analysis of an endarterectomy specimen from this patient (Panel **c**; polyclonal rabbit anti-annexin A5 antibody, ×400) shows a lesion rich in smooth muscle cells, with negligible binding of annexin A5. Panel **b**, a patient who had a left-sided transient ischemic attack (TIA) 3 days before imaging. Although this patient had clinically significant stenosis of both carotid arteries, uptake of radiolabelled annexin A5 is evident only in the culprit lesion (arrows). Immunohistology analysis of an endarterectomy specimen from this patient (Panel **d**; polyclonal rabbit anti-annexin A5 antibody, ×400) shows substantial infiltration of macrophages into the neointima, with extensive binding of annexin A5 (brown) (Kietselaer et al. 2004)

so far ^{99m}Tc-annexin A5 is one of the few tracers that have been used in the clinical setting of acute vascular disease. It should be mentioned that Annexin V is not specific for apoptosis, as many forms of vascular stress may lead to an Annexin V signal. Caspase-3-dependent pathways play also an important role in the apoptosis process. Specific targeting of caspase-3 may lead to a more specific PET signal, but this approach has not yet been validated in humans (Faust et al. 2007).

21.3.5 Angiogenesis

In atherosclerotic plaques, the formation of microvessels has been studied as a possible contributing factor to plaque destabilization and rupture. Neo-vessels in carotid artery plaques are fragile and prone to rupture and may cause plaque growth and intraplaque hemorrhage, resulting in a high-risk plaque (Takaya et al. 2005). Several angiogenic cytokines, such as vascular endothelial growth factor (VEGF), integrins,

or angiotensin, may be potential targets for molecular imaging of angiogenesis during plaque formation (Holm et al. 2009; Slart et al. 2008). Angiotensin 1 receptor (Szabo et al. 2001) and VEGF radionuclide tracers (Nagengast et al. 2007) have already been used in other diseases in which angiogenesis plays an important role, but not in atherosclerosis. For example, non-invasive imaging of angiogenesis by radionuclide VEGF tracers (Blankenberg et al. 2006) has been performed.

Hypoxic conditions in atherosclerotic angiogenesis can be imaged with radiolabelled 2-nitroimidazoles, ^{18}F-FAZA, ^{18}F-MISO, and ^{61}Cu-ATSM (Kurihara et al. 2012). To date, it is unclear if the concentration of hypoxia in plaques will be sufficient to visualize hypoxia. VEGF and VEGF-receptor expression is also induced in response to hypoxia, and therefore these VEFG receptors represent reasonable targets for imaging of mediators of ischemia-induced angiogenesis, also in atherosclerotic plaques (Virmani et al. 2005). Previous studies used ^{111}In-VEGF121 in different animal models, for example, in rabbits (Lu et al. 2003). The anti-VEGF monoclonal antibody VG76e has been labelled with the PET tracer ^{124}I, following successful validation in a mouse model (Collingridge et al. 2002). A humanized monoclonal antibody, bevacizumab, binds strongly to VEGF by the tyrosine kinase receptor and neutralizes all isoforms of the ligand VEGF-A (Ferrara and Davis-Smyth 1997; Gerber and Ferrara 2005). This makes bevacizumab also an interesting antibody to visualize angiogenesis. ^{111}In-bevacizumab and ^{89}Zr-bevacizumab reveal high uptake in vascularized tissues (Nagengast et al. 2007). It is also feasible to visualize VEGF expression within carotid endarterectomy specimens using ^{89}Zr-bevacizumab PET. ^{89}Zr-bevacizumab accumulation in plaques is specific, and tissue-to-background ratio correlates with immunohistochemistry scores (Golestani et al. 2013) (Fig. 21.6). Also radiolabelled RGD peptides as integrin alpha(v)beta3-targeted PET tracers may play an important role for the imaging of angiogenesis (Laitinen et al. 2009). Integrin alpha(v)beta3 is expressed on the plasma membrane in an active status in which it binds its ligands and transduces signals when exposed, activating external stimuli of angiogenesis in atherosclerosis such as VEGF (Laitinen et al. 2009).

Microvessels in atherosclerotic plaque can also show an overexpression of VCAM-1 and ICAM-1 (Boyle et al. 2000). However, no radiolabelled VCAM-1 tracer is produced until now. ^{64}Cu-(anti-ICAM)NPs have been used in a previous study to analyze endothelial inflammation (Rossin et al. 2008).

21.3.6 Thrombosis

In carotid artery plaques, thrombotic activity is associated with stroke and TIAs (Sayed et al. 2009). Unstable carotid artery plaques express a variety of thrombomodulatory factors, and expression of these factors is higher in unstable plaques compared to stable plaques (Spagnoli et al. 2004). In a large series of carotid endarterectomy specimens, thrombotic activity was seen in 74% and 35% of patients with

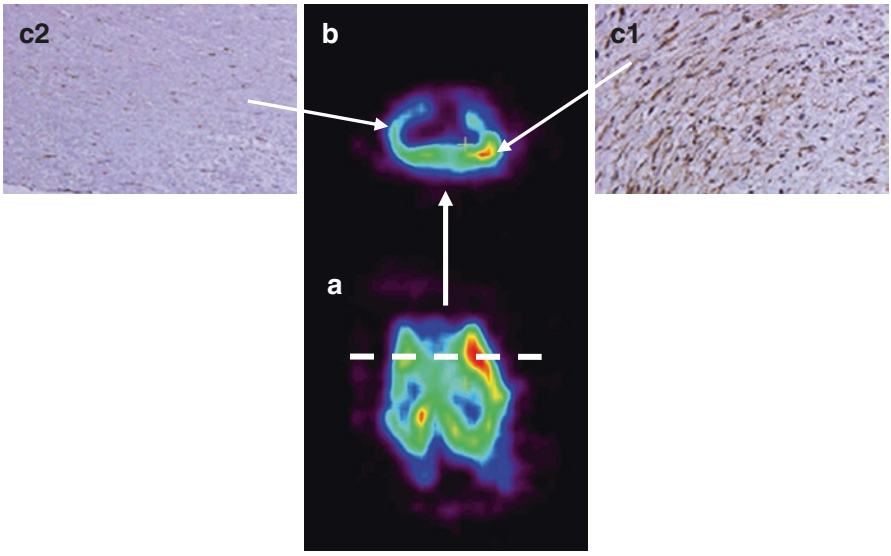

Fig. 21.6 (**a**) Coronal view of carotid endarterectomy specimen incubated with ^{89}Zr-bevacizumab using μPET. (**b**) Dashed lines show the levels of transverse views. (**c1**) Immunohistochemistry of a slide with high ^{89}Zr-bevacizumab uptake shows intense staining of VEGF (c1) in CEA specimens. (**c2**) Immunohistochemistry of a slide with low ^{89}Zr-bevacizumab uptake shows diminished VEGF staining (c2) (Golestani et al. 2013). *VEGF* vascular endothelial growth factor

ischemic stroke and TIAs, respectively, and only in 14% of asymptomatic patients. In stroke patients, thrombotic activity was seen until several months after the first cerebrovascular event (Sayed et al. 2009). These findings suggest that thrombotic activity plays a crucial role in plaque rupture and the pathogenesis of stroke (Hermus et al. 2010). Several nuclear tracers are available to image thrombus activity (Table 21.1); however most are evaluated in dated studies.

21.3.7 Plaque Calcification

Calcification may play a role in stabilizing the plaque but is also related to cardiovascular risk (Mauriello et al. 2011). The association of aortic calcification with cardiovascular events and all-cause mortality has been emphasized in several reports. Arterial calcification has been traditionally determined by CT, whereas inflammation mainly mediated by macrophage activity has been assessed by FDG-PET over the last few years. The increasing use of ^{18}F-sodium fluoride (^{18}F-NaF) PET has raised the theoretical possibility of studying active mineral deposition in the atherosclerotic plaque perhaps years or even decades earlier than they become visible on conventional imaging, such as CT. To date there have been some clinical

studies (Derlin et al. 2010, 2011a, b; Dweck et al. 2012) that have explored the feasibility of using ^{18}F-NaF PET/CT in assessing the calcification component of atherosclerosis. Derlin et al., in a retrospective study (2011a), evaluated the correlation of ^{18}F-NaF accumulation in the common carotid arteries of neurologically asymptomatic patients with cardiovascular risk factors. ^{18}F-NaF accumulation in the common carotid arteries was analyzed both qualitatively and semiquantitatively by measuring the blood-pool-corrected standardized uptake value (target-to-background ratio) and comparing it with cardiovascular risk factors and calcified plaque burden. ^{18}F-NaF uptake was observed at 141 sites in 94 (34.9%) patients. Radiotracer accumulation was colocalized with calcification in all atherosclerotic lesions. ^{18}F-NaF uptake was significantly associated with age ($P < 0.0001$), male sex ($P < 0.0001$), hypertension ($P < 0.002$), and hypercholesterolemia ($P < 0.05$). The presence of calcified plaque correlated significantly with these risk factors but also with diabetes ($P < 0.0001$), history of smoking ($P = 0.03$), and prior cardiovascular events ($P < 0.01$). There was a highly significant correlation between the presence of ^{18}F-NaF and the number of cardiovascular risk factors that were present ($r = 0.30$, $P < 0.0001$). It was concluded that carotid ^{18}F-NaF uptake is a surrogate measure of calcifying carotid plaque, correlates with cardiovascular risk factors, and is more frequent in patients with a high-risk profile for atherothrombotic events but demonstrates a weaker correlation with risk factors than does calcified plaque burden. A more recent study demonstrated comparable results in carotid patients and stated that ^{18}F-NaF PET represents a different stage in the calcification process than CT (Hop et al. 2019). They observed a similar PET assessed ^{18}F-NaF uptake and pattern in culprit and non-culprit plaques of high-risk patients, indicating that this method may be of more value in early atherosclerotic stenosis development. These studies provide a rationale to conduct further prospective studies to determine whether ^{18}F-NaF uptake can predict vascular events, or if it may be used to monitor pharmacologic therapy.

^{18}F-NaF PET/CT is also a promising new approach for the assessment of coronary artery plaque biology. Prospective studies with clinical outcomes are however needed to assess whether coronary ^{18}F-NaF uptake represents a novel marker of plaque vulnerability, recent plaque rupture, and future cardiovascular risk (Dweck et al. 2012). Active molecular calcification may be assessed by ^{18}F-NaF PET/CT much earlier than the detectable calcification identified by CT and, hence, provides clinically relevant information in the individual patient at an early stage when medical intervention is likely to have a correspondingly greater effect.

21.4 Future Perspectives

The identification of major, causal risk factors for the development of atherosclerosis provides physicians reliable tools to identify individuals who are at high risk for atherosclerosis-related clinical events. However, the quantitative risk assessment would be significantly improved if atherosclerotic plaque vulnerability could

be assessed more directly. The emerging area of nuclear imaging techniques can provide measures to evaluate the vulnerability of atherosclerotic plaques, thereby improving the prediction of clinical events. Important is the need to learn which of the different morphological, molecular, biological, or mechanical features of vulnerable plaques are clinically relevant to the outcome of patients. It is likely that more than one plaque feature will be needed to make a clinically useful assessment. In this regard, combined imaging will be necessary. SPECT/CT and PET/CT offer the possibility to combine morphology and pathophysiological processes. PET and SPECT are able to accurately detect the tracer in very low concentrations (pg/mL). Anatomical detail is however warranted; therefore effort must be invested to reach an optimal resolution. Clinical PET has a far better resolution than SPECT and is in matter of quality a method of first choice but more expensive. A more recent study highlights the importance of standardizing all aspects of methodological approaches to ensure accuracy and reproducibility (Giannotti et al. 2017). They found statistically significant differences in FDG SUV measurements in 101 carotid patients between 2 software packages, ranging from 9% to 21.8%, depending on ROI location. In 79% ($n = 23$) of the ROI locations, the differences between the SUV measurements from each software package were found to be statistically significant. The time taken to perform the analyses and export data from the software packages also varied considerably. Physicians must be aware that when a PET-CT data set is analyzed, with the same software package should be used or cross-calibration between packages should be performed. The use of radionuclide tracers is associated with ionizing radiation, and this potentially may limit their clinical application. Much effort has been directed toward reducing the radiation dose and the effective dose equivalent, and organ doses are now comparable or lower than the radiation dose used in CT examinations, depending on the CT acquisition protocol (Huang et al. 2009). With modern equipment even lower activities of the tracer are required, and a safe total radiation dose can be achieved. For molecular imaging, MRI can also be used in conjunction with PET, using a hybrid PET-MRI system (Pichler et al. 2010). In contrast to PET and CT, MRI offers excellent soft-tissue contrast in combination with high spatial resolution, but it gives little functional information. Simultaneous acquisition of PET and MRI under identical physiological circumstances and optimal matching of both images could overcome the shortcomings of both separate techniques. They can provide quantitative functional information about the atherosclerotic lesion, with more accurate localization and motion correction. Taken together, this could improve the overall differential diagnosis and guide treatment strategies, resulting in better medical care. MRI allows for the characterization of plaque composition, i.e., the discrimination of lipid core, fibrosis, and intraplaque hemorrhage deposits; however identification of calcification is difficult. A further important focus of research is the development of specific MRI probes (targeted molecular imaging), which can be combined with PET probes to obtain complementary information simultaneously. The combination of different modalities (PET and MRI), imaging different targets, allows useful synergies for non-invasive in vivo imaging. Molecular

MRI probes for fibrin, macrophages, and high-density lipoprotein (HDL) have been tested at the preclinical level to visualize thrombotic material, inflammation, or transport of HDL metabolism within atherosclerotic plaques in vivo (Frias et al. 2004; Skajaa et al. 2010). A variety of magnetic nanoparticles (MNPs) have been developed to detect aspects of inflammation in the atherosclerotic plaque. Nanoparticles are digested by macrophages and accumulate in macrophages that are present in the carotid artery plaque. MNPs are detectable in vivo by high-resolution MRI. The ability to image the presence or biological activity of specific molecules ("molecular imaging") in atherosclerotic plaques in vivo is of considerable interest. Assessment of molecular information in vivo requires high-affinity, target-specific contrast agents, with marked signal amplification, and high-resolution imaging modalities, such as magnetic resonance. Most of the available paramagnetic magnetic MRI contrast agent constructs, however, are not capable of delivering a large amount of gadolinium ions to induce a large MRI signal. Moreover, some of the MRI contrast agent molecules may be too large to have free access to biochemical epitopes within the vascular subendothelium of atherosclerotic plaques. The role of specific enhancers deserves therefore further investigation (Corti and Fuster 2011).

Another new and promising technique to image biological plaque activity in atherosclerotic plaques is near-infrared fluorescence (NIRF) imaging. This technique operates in the near-infrared spectrum of light and uses activatable probes that provide fluorescent images to detect enzymatic action of proteolytic enzymes such as MMPs and CCPs. Probes, such as gelatinase-activated (Deguchi et al. 2006) or cathepsin B-sensitive agents (Chen et al. 2002), are conjugated with NIRF fluorochromes. These probes produce very low background fluorescence and after activation produce a profound amplification of fluorescence. This way, vulnerable plaques with high proteolytic activity can be identified—at this stage only ex vivo or in animal studies. The first NIRF application in human carotid artery plaques was reported by Wallis de Vries et al. who detected MMP-2 and MMP-9 in a carotid endarterectomy specimen (Fig. 21.7) (Wallis de Vries et al. 2009). Other targets, like the inflammatory molecule vascular cell adhesion molecule-1 (VCAM-1) receptor expression in carotid artery plaques, can be imaged in vivo with fluorescence imaging, but their value is unknown (Nahrendorf et al. 2006). It will take technical developments and preclinical toxicity testing before in vivo studies in humans can be performed with NIRF. Furthermore, the reporter capabilities and specific cellular distribution in vivo need to be understood, and technical aspects, such as dose-finding studies of a fluorescent tracer and timing of imaging, need to be improved to obtain optimal fluorescence signals of a plaque in situ.

In conclusion, several radiopharmaceuticals are available to image different processes of the vulnerable plaque. Hybrid imaging will increase the accuracy of plaque identification. New technique developments will support this.

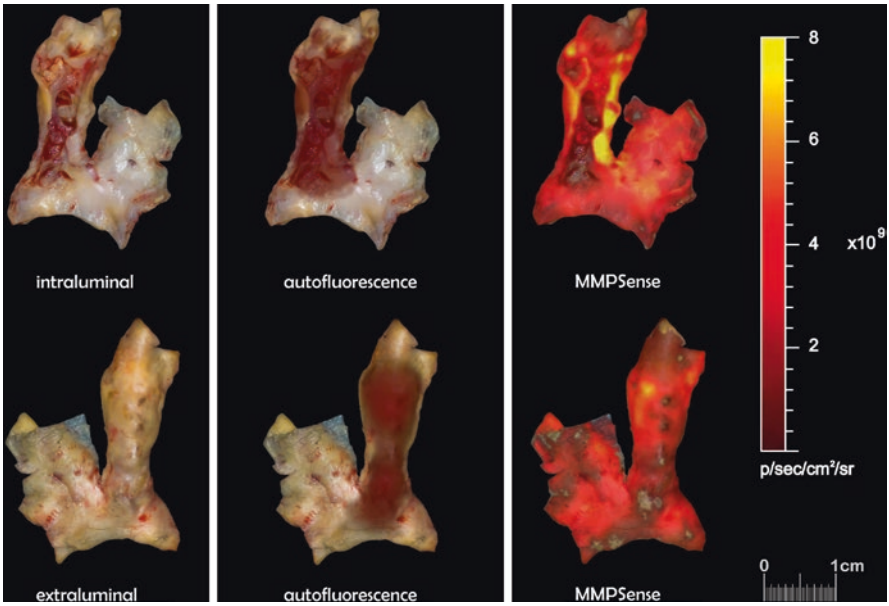

Fig. 21.7 MMPSense 680 fluorescence probe enables mapping of differential MMP activity in endarterectomized atherosclerotic plaques. Carotid endarterectomy specimen in white light (left), near-infrared fluorescence signal before (autofluorescence, middle) and after incubation with MMP-sensitive activatable fluorescent probe (MMPSense, right) within the IVIS Spectrum (bioluminescence and fluorescence in vivo imaging system). After incubation with MMPSense, highlighted hot spots were identified both at the intraluminal and extraluminal side, most present in the origin of the internal carotid artery and at the level of the common carotid bulb. Spots indicated by the frames were excised and processed for in situ zymography, real-time reverse-transcriptase polymerase chain reaction, and morphological analysis. The intensity of the NIRF signals obtained throughout the plaque is expressed as number of photons/s/cm^2/steradian (Wallis de Vries et al. 2009)

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