# Imaging biomarkers of carotid vulnerable plaque for stroke risk prediction and their potential clinical implications

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## ABBREVIATIONS

18F-FDG PET: fludeoxyglucose 18F - PET

CE: Contrast-enhancement

CEUS: Contrast Enhanced Ultrasound

CT: Computed Tomography

CTA: Computed Tomography Angiography

DCE-MRI: Dynamic Contrast Enhancement Magnetic Resonance Imaging

DECT: Dual Energy Computed Tomography

DSA: Digital Subtraction Angiography

DWI: Diffusion Weighted Imaging

FC: Fibrous Cap

IPH: Intra-plaque Haemorrhage

IR-FSPGR: Inversion Recovery Fast Spoiled Gradient Recalled Acquisition in the Steady State

IR-TFE: Inversion Recovery Turbo field Echo

IPN: Intra-plaque Neovascularization

LRNC: Lipid-rich Necrotic Core

MRA: Magnetic Resonance Angiography

MRI: Magnetic Resonance Imaging

MPT: Maximum Plaque Thickness

NASCET: North American Symptomatic Carotid Endarterectomy Trial

NM: Nuclear Medicine

PET: Positron Emission Tomography

TIA: Transient Ischemic Attack

US: Ultrasound

## ABSTRACT

Stroke represents a massive public health problem and current European and American guidelines for prevention of stroke in patients with carotid plaques are based on the quantification of the percent-reduction in luminal diameter due to the atherosclerotic process. However, better strategies for prevention of stroke are required because evidence has shown that some sub-types of plaques, so-called vulnerable (plaques that have a high likelihood to cause stroke, independent of the degree of stenosis),can predict the likely occurrence of stroke independent of the degree of stenosis. Advances in imaging techniques have allowed for the routine characterization and detection of carotid plaque features of vulnerability. In particular, intra-plaque-haemorrhage is accepted by neurologist and radiologists as one of the identifying features of vulnerable plaque but also other features such as plaque volume, neovascularization, and inflammation seem to be promising to be considered as biomarker of vulnerability even if further confirmatory studies are necessary.

# 1-INTRODUCTION

Stroke represents a massive public health problem and approximately 18-25% of all ischemic strokes are due to thromboembolism caused by carotid atherosclerotic disease[1]. Current European and American guidelines for prevention of stroke in patients with carotid plaques are based on the quantification of the degree of stenosis[2,3] and this parameter is currently considered the key element for stratifying the severity of carotid artery atherosclerosis and for the choice of strategies to prevent the occurrence of stroke.

The evolution of imaging techniques has allowed for the routine characterization of carotid plaque features and the traditional concept of using degree of luminal stenosis as the sole imaging marker for the selection of the optimal therapeutic approach is challenged by a growing body of evidence demonstrating that some types of carotid plaques, so-called "vulnerable carotid plaques", have a high likelihood to cause ischaemic stroke, independent of the degree of stenosis[4,5,6]. Vulnerable plaques are defined as atherosclerotic plaques that have a high likelihood to cause thrombotic complications[4]. Plaques that tend to progress rapidly are also considered to be vulnerable[7].

Currently there is a debate among neurologist, neuroradiologist, vascular surgeons and neurosurgeons regarding the clinical impact of the vulnerable plaques and their implications for treatment and outcome because in the past years the degree of stenosis was considered the lead parameter for the choice of the therapeutic option, but nowadays several evidences have showed that the carotid plaque composition plays a role. This paradigm shift (from stenosis degree to plaque) represents an important element for research in primary and recurrent prevention of ischemic stroke because of its potential implication for the management of the patient and there is an increasing need for better diagnostic and therapeutic strategies as highlighted in current guidelines of American Society of Neuroradiology and European Society of Cardiology[8,9]. The American Society of Neuroradiology (ASNR) Vessel Wall Imaging Study Group[8] published in 2018 the Carotid Artery Imaging Wall Perspective and Guidelines which focused on the

technological implications and impact of technologies for carotid plaque imaging. In the same year, the European Society of Cardiology[9] recommended that carotid artery revascularization should be considered for *asymptomatic* patients with a life expectancy >5 years and 60-99% carotid artery stenosis and *imaging features of plaque vulnerability* by showing that the scientific community is accepting that the risk of stroke, carotid plaque related , is not only due to the degree of stenosis but also plaque composition.

The Review critically discusses the developments in the assessment of imaging biomarkers of carotid vulnerable plaque, compare relative strengths and limitations of the plaque imaging modalities, provide data of their predictive value of plaque imaging in patients with symptomatic and asymptomatic plaque (with and without stenosis), add prevention aspect and discuss future research directions.

## 2-CAROTID PLAQUE FEATURES OF VULNERABILITY

The aim of plaque imaging is to look beyond the lumen (and the stenosis degree) and to identify those imaging biomarkers of carotid vulnerable plaque that are best suited for stroke risk prediction[4,6] (**Table 1**). In the following six sections the features linked to plaque vulnerability are presented based on most evidence (**Figure 1**).

### 2.1Intraplaque haemorrhage

Intra-plaque haemorrhage (IPH) is one of the key features of carotid vulnerable plaque[10], as well as a contributor to enlargement of the lipid-rich necrotic core (LRNC) and more rapid plaque progression[11]. A meta-analysis of 9 studies indicates that MRI detection of carotid IPH is associated with increased risk for future ischemic stroke in patients with symptomatic and asymptomatic carotid stenosis[12] (HR =4.59; 95% confidence interval, 2.91-7.24). IPH is also more prevalent in carotid arteries ipsilateral to embolic strokes of undetermined source[13] even if other causes could be

considered such as the retrograde flow[14]. IPHs can occur bilaterally and this could explain bilateral lesion detected in brain MRI due to carotid atherosclerosis rather than a cardio-embolic source[15].

IPH is considered the strongest imaging parameter associated with the occurrence of stroke[16]. MRI is the best imaging technique for the detection of IPH because the appearance of IPH depends on the oxidative state of hemoglobin[17] and can be easily detected using commonly used imaging sequences (T1-weighted fat saturated TSE [T1-TSE fs] Inversion-Recovery Turbo-field-Echo [IR-TFE] or Inversion-Recovery Fast-Spoiled Gradient Recalled Acquisition in the Steady-State [IR-FSPGR])[8]. A prospective study showed that in MRI carotid plaque imaging it is not necessary to use dedicated carotid small field-of-view (FOV) surface coils for IPH detection since this can be achieved at lower spatial resolution using large FOV neck coils[18]. It is important to note that MRI allows categorization of IPH into fresh (type 1), recent (type 2), and old (type 3) subtypes but that there is currently no evidence correlating the subtype of IPH with an increased or reduced occurrence of future ischemic events[15].

Ultrasound (US) and CT are less suitable for detection of IPH. US has low sensitivity and specificity for the detection of IPH[19] and CT shows conflicting results as CT has difficulties to differentiate between fibrous, lipid and IPH due to an overlap of Hounsfield Units (HU) values[20] (The HU is a way to characterize radiation attenuation in different tissues).

### 2.2 Lipid-Rich Necrotic Core and Fibrous cap

Evidence supports that LRNC, an heterogeneous tissue composed by cholesterol crystal, debris of apoptotic cells and particles of calcium, in carotid plaques is predictive of an increased risk of a stroke[12]. A longitudinal MRI study of 120 asymptomatic subjects showed that carotid plaques with a maximum percentage of LRNC (%LRNC) greater than 40 (where % LRNC = LRNC area/wall-area) were more likely to develop Fibrous Cap (FC) rupture during follow-up (3 years) compared to the subjects with %LRNC < 40%[21]. However, there were too few events in this study to assess whether %LRNC was associated with stroke.

Both CT and MRI can identify the presence of lipid components thanks to lipid-tissue attenuation properties and signal characteristics[22-25]. However, MRI is superior compared to CT in the detection of the LRNC because this technique can distinguish between LRNC and IPH whereas in CT both of these two features show attenuation values < 60HU[26]. A cross-sectional study has demonstrated that the presence of hypoechogenic plaque areas on US is associated with the LRNC, in particular, echolucent areas near the plaque surface (so-called juxta-luminal-black-areas)[27]. Currently, US cannot be considered reliable in the detection of LRNC because it is very difficult to distinguish LRNC from IPH (both appear hypoechogenic)[27].

The FC is a layer of fibrous connective tissue which separates the core of the plaque from the arterial lumen. FC alterations (thin or ruptured cap) are considered an important feature of plaque vulnerability[12,28]. MRI is considered the preferred technique to image this feature[29,30], especially with the use of gadolinium-based contrast agents[31,32].

### 2.3Plaque Inflammation and neovascularization

Another feature of plaque vulnerability is inflammation, which is often associated with angiogenesis and referred to as plaque activity[33]. In a cross-sectional study of 62 subjects a correlation between macrophage plaque infiltration, plaque rupture and ischemic symptoms was found[34]. Inflammatory cells accumulate in specific areas of the plaque, typically the shoulder or in the FC[28,31]. Imaging of inflammation is currently in the domain of research and not routinely used in the clinical practice. In the last five years, several studies have demonstrated the potential of PET to image and quantify plaque inflammation[35-38]. However, there is currently no consensus on the methodology for quantification of fludeoxyglucose-18F (18F-FDG)-uptake to image inflammation in patients with atherosclerosis[39]. Detection of intra-plaque inflammation with use of MRI showed a correlation between histologic markers of inflammation suggesting that MRI could be a quantitative and noninvasive marker of plaque inflammation[40].

Molecular imaging is a promising imaging technique for the detection of plaque's inflammation. Several nanoparticles (eg, iron oxide, sodium fluoride, Polyethylene glycol molecules) are being used for molecular imaging of atherosclerosis in human and animal models[41-43]. In particular iron oxide MRI contrast agents provide highly efficient iron-labeling in macrophages for MRI–based-detection in vivo and were reported as very promising in the detection of plaque inflammation[42]. In a cross-sectional study of 23 patients, PET-$^{18}$F-sodium fluoride was also used to distinguish between vulnerable and non-vulnerable human carotid plaques[43]. But Molecular imaging has the limit that it will require a relative long delay (2- 24 hours) between the time of contrast injection and post-contrast imaging[41-43] making this type of procedure much more complex compared to CT or MRI.

Another important feature of plaque vulnerability is intra-plaque neovascularization that is associated to the activity of the plaque in terms of increased risk of neovessel rupture and haemorrhage and inflammation[44]. Inflammation and neovascularization might be also associated with stroke, but evidence is inclusive[44]. A cross-sectional study of 175 individuals has shown that plaque enhancement on Contrast-Enhanced US (CEUS), a sonographic technique where microbubble contrast agents filled with a perfluorinated gas are injected as intravascular tracers, is statistically associated with intra-plaque neovascularization[45]. Similar results were obtained in a study on 27 patients[46] and these findings were confirmed in a meta-analysis of 20 studies published in 2016[47] which concluded that CEUS is a promising technique to detect intra-plaque neovascularization. In another cross-sectional study of 41 subjects performed with CEUS, a positive correlation was found between the micro-embolic signals and the presence of neo-vascularization in patients with symptomatic atherosclerotic carotid plaque[48]. CT can also help with the detection of intra-plaque neovascularization and in its quantification as the amount of contrast enhancement on CT is associated with the extent of neovascularization[49].

Detection of intra-plaque neovascularization with use of MRI showed a correlation between the degree of plaque enhancement and the degree of neovascularization[50]. Dynamic Contrast

Enhancement MRI (DCE-MRI) perfusion imaging measures the changes of the signal in tissues over time (usually up to 5-10 minutes) after bolus administration of gadolinium and permits quantification of plaque vascularity[51]. However, one of the main limitations of DCE-MRI is that the vessel wall is difficult to image dynamically because of its small size and motion artifacts[51].

### 2.4Carotid artery plaque thickness

Nowadays the thickness of the carotid artery plaque is easily quantifiable[52,53] with US, CT and MR and the Maximum Plaque thickness (MPT) represents the maximum thickness of the plaque. According to the Mannheim consensus, plaques are defined as having a thickness higher than 1.5 mm[54]. In a MRI cross-sectional study of 1072 subjects, the MPT was more strongly associated with cerebral ischemic symptoms than was the degree of stenosis[55], demonstrating that plaque size represents a parameter associated with the occurrence of stroke.

### 2.5Surface Morphology

In the past years before to reach the technology necessary to observe the carotid plaque structure, one of the parameter assessed was the surface morphology of the plaque The surface of the plaque can be categorized as smooth, irregular (plaques whose surface fluctuates from 0.3 mm to 0.9) or ulcerated (reserved for cavities measuring at least 1 mm)[56]. The irregular morphology of the luminal surface, and in particular the presence of ulceration, are considered risk features for stroke[56].

Carotid plaque surface assessment can be performed by US, CT and MRI with varying levels of diagnostic accuracy. Although some authors do not consider US an optimal technique for the detection of irregular plaque surface and ulcerations because of the acoustic shadowing of calcified components[57,58], it has been shown that CEUS can be effective for this purpose by improving the detection accuracy because microbubbles facilitate the differentiation between the intimal layer and the blood-flow[59]. As demonstrated in two cross-sectional studies of 237 and 600,

CT and MRI respectively (in particular with the use of contrast material[60]) offer optimal diagnostic accuracy for detecting ulcers with performance superior to that of US (CT sensitivity > 90% versus US < 40%)[58].

The characterization of the surface morphology with the presence of ulceration is a further basic feature of plaque vulnerability, however the predictive value of this feature is debated because some authors suggest that ulceration is a marker of previous plaque rupture even if it can be also an influential predictor of occurrence of future ischemic stroke[61].

### 2.6 Carotid plaque volume

A longitudinal study of 62 subjects using CT showed that the volume of the carotid artery plaque is associated with vulnerability of the plaque[62] and another cross-sectional study of 70 individuals showed that the volume of the carotid artery plaque is associated with presence of stroke[63]. Because of the excellent spatial resolution of CT, it is possible to calculate accurately the total plaque volume and also the volume of the sub-components of the plaque (fatty – mixed – calcified) according to the attenuation values of the voxels[64]. A prospective longitudinal study in 63 patients (follow-up 55 months) has demonstrated that the annual progression of carotid plaque volume is independently associated with recurrent ischemic stroke[65]. Similarly, MRI is proven to be highly useful for plaque component volume quantification[66,67] even though the spatial resolution of MRI is lower than that of CT, but its soft tissue contrast is superior. A meta-analysis on 7 studies on 3D US suggested a good reproducibility for the evaluation of carotid plaque volume[68].

## 3-PREVENTION OF STROKE

The efficacy of carotid revascularization in prevention of recurrent stroke in symptomatic patients (patient who previously suffered a Transient Ischemic Attack- TIA - or stroke) with

moderate (50-69%) or severe (70-99%) carotid stenosis is well documented but a study of 853 patients showed that 89.7% (44/49) of subjects with symptomatic with moderate or severe stenosis who remain untreated did not have a recurrent stroke at 5 years[69]. Therefore, plaque imaging could play a role in identifying those patients that have stable plaques and in which a carotid intervention might not be necessary. In addition, plaque imaging could help to identify symptomatic patients with mild (<50%) stenosis with vulnerable plaques that are at high risk of recurrent stroke and which could benefit from carotid intervention.

A meta-analysis on 5 randomized controlled Trial (3019 subjects) has shown a modest but significant benefit for carotid intervention in asymptomatic patients with severe carotid stenosis[70] but in another meta-analysis on 47 studies the summary incidence of ipsilateral stroke across 26 cohorts receiving medical therapy alone was only 1.68% per year[71]. Therefore, it is no longer clear that the moderate benefit of carotid endarterectomy in preventing stroke seen in earlier trials is still present in the context of modern medical therapy[71]: it seems crucial to identify patients with asymptomatic carotid stenosis with stable and with unstable plaques and to select those patients which might benefit from a carotid intervention.

### 3.1- Prediction of recurrent stroke risk in patients with symptomatic carotid stenosis

Patients with symptomatic carotid stenosis are currently considered candidates for revascularization in order to avoid the occurrence of a recurrent stroke[9]. The risk of stroke during the first 90 days after a TIA is between 3.7% and 11.7%[72,73]. The presence of plaque features of vulnerability (IPH, LRNC, Status of the FC) can further increase the risk of occurrence of ischemic events.

Two meta-analysis[12,16] of 9 and 8 prospective studies respectively have shown a strong link between the presence of IPH and the occurrence of future ischemic stroke in patients with symptomatic carotid stenosis. Therefore, in patients symptomatic with carotid stenosis and

detection of IPH a procedure of revascularization should be warranted. Absence of IPH within the plaque seems to be associated with a benign clinical course, even amongst patients with symptomatic moderate or severe carotid stenosis[74].

There are also plaque features associated with low risk recurrent stroke in subjects with severe degree of stenosis such as the heavily calcified plaque[75]. A cross-sectional meta-analysis[76] of 16 studies found a significant negative relationship between calcified plaque and ipsilateral stroke (OR, 0.5; 95% CI, 0.4-0.7). CT-based assessment of calcium content can be performed semi-quantitatively using calcium scores[77], or quantitatively with direct volume plaque components analysis[8,78].

However, the impact of calcium into the carotid artery plaque could be more complex: a recent study performed on 229 carotid plaques identified two types of calcium salts in atheromatous plaques, hydroxyapatite and calcium oxalate and an association between hydroxyapatite calcification and vulnerable plaques was found whereas calcium oxalate calcifications were mainly detected in non-vulnerable plaques[79]. This finding could further increase the utilization of multi-energy CT scanners because of their potential to perform spectral analysis and distinguish between hydroxyapatite and calcium oxalate calcifications[80].

### 3.2-Prediction of primary stroke risk in patients with asymptomatic carotid stenosis

The prevention of primary stroke in patients with asymptomatic with high risk carotid plaques is most challenging due to the risk of rupture independent of the degree-of-stenosis[21,81]. A prospective longitudinal study with 154 asymptomatic patients with 50-79% carotid stenosis, followed by MRI for mean follow-up period of 38.2 months, showed that carotid plaques with features of vulnerability were associated with subsequent stroke[82] (thinned or ruptured FCs = HR 17, p = 0.001 / IPH = HR 2.6; p = 0.006 / larger-maximum %-LRNC = HR 1.6; p = 0.004 / larger MWT = HR 1.6; p = 0.008).

In a longitudinal MRI cohort study of 1,190 patients with asymptomatic carotid stenosis with mean follow-up of 53 months[83], IPH was shown to be a high-risk factor for a subsequent stroke event with a significantly lower event-free survival rate in the high-signal-intensity group (HR 4.2; 95% CI 1.0-17.1; p = 0.04). In another longitudinal MRI study[84], the plaques of 198 patients were followed for 4-years and an increase in IPH prevalence with age and hypertension was reported, highlighting the importance of blood-pressure lowering to prevent stroke[85,86].

Results from these studies suggest that it is possible to detect imaging features (IPH, thin/ruptured FC, %LRNC, larger MWT) with predictive value for stroke occurrence also in patients that had not previously suffered from TIA. Incorporating the findings from these studies with the emerging concepts of plaque regression[87] (overall reduction in plaque volume) and the results of lipid-lowering therapy and anti-inflammatory therapy[88,89] could help build strategies combining imaging biomarkers in follow-up analysis to monitor drug effects.

A longitudinal (median follow-up of 35.1 months) MRI study, involving 232 patients with atherosclerotic disease, revealed that the amount of lipids into the carotid plaque and FC status are significantly correlated not only with ischemic stroke but also with systemic cardiovascular outcomes (fatal and nonfatal myocardial infarction, ischemic stroke, hospitalization for acute coronary syndrome) and that biomarkers of carotid plaque vulnerability could be used as novel surrogate markers, not only for stroke, but for systemic athero-thrombotic risk[90,91].

### 3.3-Identifying high-risk plaque features in patients with non-stenotic plaques

While sub-stenotic plaque in coronary arteries is a well-recognized cause of myocardial infarction[92], the role of sub-stenotic plaques in carotid arteries as a cause of stroke requires further research. Growing evidence suggesting that stroke may be caused by presence of vulnerable carotid artery plaques even in the absence of moderate/severe stenosis (>50%)[6,55,81,93] and there is growing debate for the role of some features (IPH, %LRNC) in this type of patients but currently weak evidence with the future occurrence of ischemic events can be definitively considered. Further

secondary analysis from ongoing prospective trials assessing the impact of plaque components versus stroke occurrence also in subjects with sub-stenotic carotid arteries (CREST/NCT02089217; ECST-2/ISRCTN97744893, ACAS-2 /ISRCTN21144362) could help to confirm or exclude other parameters.

Mild stenosis (< 50%) associated with plaque vulnerability is also linked to the concept of positive plaque remodeling[94]. This condition occurs when progression of a carotid plaque leads to outward expansion of the outer wall boundary, due to the increase in plaque volume, while preserving the dimension of the lumen[94]. The fact that features of vulnerability can be found in plaques with mild stenosis[55] and in some cases in the absence of any detectable stenosis could be explained with the positive remodeling of the plaque. Under this scenario, a plaque with relatively little luminal stenosis can be disproportionately advanced based on its composition due to outward growth. It has been hypothesized that plaque thickness and normalized wall index may be a better indicator of the severity of atherosclerotic disease than the degree of stenosis[95] but this hypothesis cannot be considered yet confirmed until proven in controlled trials. It is possible that if a patient suffers from a stroke ipsilateral to a carotid vulnerable plaque, the patient may warrant carotid revascularization (or intensified medical therapy) even if stenosis thresholds defined by NASCET criteria are not met[56].

### 3.4-Longitudinal Assessment of atherosclerotic plaques

Longitudinal study has demonstrated the progression of the carotid artery plaque and in particular the expansion of IPH volume is associated with an increased occurrence of stroke[84] (**Table 2**). It has also been shown that Intima-medi-Thickness[96] and plaque progression, measured by US, increases stroke risk in patients with asymptomatic carotid stenosis[97]. Moreover, while plaque atherosclerosis has often been considered as a chronic and irreversible disease process, a meta-analysis of 7 studies provided evidence that atherosclerosis can regress[87] with high-dose lipid-lowering therapy [**Figure 2**]. In addition, high-dosage statins beneficially influence the composition

of carotid atherosclerosis by shifting the composition from vulnerable plaque with a lipid core to a more stable calcified plaque, as demonstrated in the longitudinal Rotterdam Study in 1,740 subjects who underwent carotid MRI[98]. Another meta-analysis of 9 studies provided evidence that a significant interaction between changes in levels of cholesterol, C-reactive protein, increase of carotid plaque echogenicity and the benefits of statins on atherosclerotic plaque regression[99].

The Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial of 10,061 subjects[88] showed that the use of anti-inflammatory therapy targeting the interleukin-1β innate immunity pathway determined a significantly lower rate of cardiovascular events compared to placebo. These results indicate that intensive medical (lipid-lowering and anti-inflammatory) therapies may drive plaque reversion and conversion to a stable phenotype.

## 4-CONCLUSION AND FUTURE DIRECTION

The identification of imaging biomarkers related to an increased or decreased risk of occurrence of stroke represents a fundamental parameter for the prevention of ischemic stroke.

Several imaging techniques can be used to explore the carotid artery plaques and the features of vulnerability and the information offered are in some cases complementary to each other. Currently, US, because of its wide availability and low cost, is primarily used in assessing the plaque's echogenicity with good sensitivity in the detection and characterization of vulnerable carotid plaques[100-103] but its accuracy - compared to CT and MRI - is sub-optimal[104]; in addition, scarcity of consistent inter- and intra-observer agreement and poor signal-to-noise ratio limit the use of this technique[100]. Furthermore, the operator-dependent nature of US (more than the other imaging techniques) renders longitudinal monitoring difficult[100]. CT allows assessment of the burden (volume) of atherosclerotic plaque and detection of ulcerations[8], with good detail in the morphological analysis and for the calcium identification[8] but the limitations are mainly related to the radiation dose delivered to the patients and to the potential side effects of contrast materials

(contrast-induced renal failure; hypotension; bronchospasm). Moreover, CT has difficulties to reliably differentiate between the soft plaque components due to an overlap in HU values and is unable to identify the FC and determines overestimation of the stenosis grade due to calcium deposit[105]. MRI is currently the most suitable imaging technique to characterize features of plaque vulnerability. Among the features that can be detected, the literature clearly shows that IPH has strong association with the occurrence of future stroke[68,69]. We support the motion of adding an IPH-detecting vessel wall sequence to the standard MRI examination of the brain, which only adds 4-6 minutes scan time and can be performed using standard clinical coils, making clinical translation of this feature feasible and achievable[107]. Drawbacks of MRI are the relatively longer overall study time, and sensitivity of image quality to motion effects[106].

It is important to underline that new developments in imaging techniques (e.g. CEUS for plaque neovascularization, CT for IPH detection, neurovascular coils for MRI plaque imaging, DCE for plaque vascularity, 18F-FCH for plaque inflammation)[39,44,49] cannot be considered yet as mainstream techniques for plaque imaging or as state of the art techniques. The suggestion that these techniques can be used already in clinical practice is premature as it is unclear whether they can improve treatment strategies and ultimately their effects on outcomes have not been thoroughly investigated. Moreover, it is also important to remember that there are some technical requirements to perform optimal plaque imaging (**Table 3**)[8].

Evidence indicates that treatment decision based on plaque features could be beneficial in terms of cost-effectiveness. Cost effectiveness analysis aims to identify the best approach including economic impact and balancing the advantages with regard to risk prevention and related direct costs. In a model-analysis study, two competing stroke prevention strategies were compared: a medical strategy (intensive medical therapy-based management) versus an imaging-based strategy (imaging-based strategy in which the subset of patients with asymptomatic carotid artery stenosis with IPH on MR images would undergo immediate carotid endarterectomy in addition to ongoing intensive medical therapy). It has been shown that MRI-IPH imaging can be used as a cost-effective

tool to identify patients with asymptomatic carotid artery stenosis most likely to benefit from carotid endarterectomy[108] with subsequent impact on life expectancy (12.95 years vs 12.65 years) and economic ($13,699 vs $15,297).

In the next future some challenges need to be clarified. In particular, a key point is to demonstrate the link between biomarkers of plaque vulnerability and their role on clinical decision making on the outcome. Several prospective studies with some preliminary results or rationale and design have already been published (MESA[109], ARIC[110], SCAPIS[111], CAPIAS[112], PARISK[113], CAIN[114],Rotterdam Scan Study[115], CARE-II[116], HeCES2[117]). These studies aim to assess the value of plaque imaging in stroke risk stratification by showing that the identification of vulnerable plaque with MRI aids in ischemic stroke prediction and improves the reclassification of baseline cardiovascular risk. Several ongoing randomized clinical trials (SmartRisk, NCT00860184; CREST-2, NCT02240862; ACST-2, ISRCTN21144362) are also assessing the value of plaque imaging in stroke risk stratification and outcome. Ongoing randomized trials compare best medical therapy alone versus carotid revascularization either select patients (such as ACTRIS-NCT02841098), or allow to measure the benefit of revascularization (such as ECST-2 - ISRCTN97744893) based on carotid plaque MRI or other extended imaging (**Table 4**).

Another challenge is to define among the many different features of vulnerability those that are best suited to identify the best therapy for each individual patient and which help to obtain an optimized risk model which goes beyond the degree of stenosis and which incorporates the morphology and composition of atherosclerotic plaques. Regarding this last point Artificial intelligence (AI) could play a fundamental role. Recent advances in the field of AI have opened up new avenues for creating novel modeling and predictive methods for clinical use. The explosion of imaging data is creating a path for such approaches because of the huge amount of information included in CT and MRI data sets. Deep learning may provide the ability to identify patterns of imaging information and improve risk stratification[118] with the automated detection of those

quantitative biomarkers by automatically creating a model-of-risk incorporating all the imaging features from different techniques using a multi-technique/features approach[119].

Finally, further evaluation in randomized clinical trials is needed to establish the exact role of vulnerable plaque biomarkers in clinical decision-making for the prevention of ischemic stroke. Awaiting the results of such trials, carotid plaque imaging may be beneficial at present because the presence of some detectable features is associated with a higher risk of future strokes and may warrant closer clinical follow-up and consideration for more intensive medical therapy or – in selected patients - even revascularization.

## SEARCH STRATEGY AND SELECTION CRITERIA

References for this review were identified by searching PubMed (for articles published between Jan 1st, 2013 and December 31th, 2018. Search terms included "Carotid", "Plaque", "Imaging", "Inflammation", "CT", "CTA", "MR", "MRA", "US","CEUS", "PET", and "Molecular Imaging". In addition, the reference lists for the identified studies were reviewed and evaluated to identify additional articles. There were no language restrictions. The final reference list was generated on the basis of originality and relevance to the broad scope of this Review and preference was dedicated in the inclusion of controlled trials, longitudinal studies, meta-analysis and studies with adequate methodology. In addition, published practice guidelines and their reference lists were reviewed.

## Acknowledgments

The authors would like to thank Dr Vasileios Rafailidis for his help.

## Declaration of interest section

- Dr Saba has nothing to disclose
- Dr Saam has nothing to disclose
- Dr Jager has nothing to disclose
- Dr Yuan has nothing to disclose
- Dr. Hatsukami reports grants from Philips Healthcare, outside the submitted work;
- Dr Saloner has nothing to disclose
- Dr Wasserman has nothing to disclose
- Dr. Bonati reports grants from Swiss National Science Foundation, grants from University of Basel, grants from Swiss Heart Foundation, during the conduct of the study; personal fees and non-financial support from Amgen, grants from AstraZeneca, personal fees and non-financial support from Bayer, personal fees from Bristol-Myers Squibb, personal fees from Claret Medical, outside the submitted work;
- Dr. Wintermark reports fees (equities) for the following companies:
    - MoreHealth: (equity) second opinion service, no relevance to the topic of the paper
    - Magnetic Insight: (equity) imaging technique for rodents, no relevance to the topic of the paper (technique not mentioned in the article)
    - Icometrix: (equity) multiple sclerosis focus, no relevance to the topic of the paper

## Author contribution section

- Luca Saba: literature search, figures, writing
- Tobias Saam: literature search, writing
- H R Jäger: literature search, writing; figures

- Chun Yuan: literature search, writing
- Thomas S. Hatsukami: literature search, writing
- David Saloner: literature search, writing
- Bruce A. Wasserman: literature search, writing
- Leo Bonati: literature search, writing
- Max Wintermark: literature search, writing

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## Tables

## FIGURE LEGENDS

**Figure 1 Imaging features of plaque vulnerability**

Example of the features of carotid plaque vulnerability obtained with the different imaging technologies: CT, MRI (3T) and US imaging. In the columns are categorized 6 types of features of vulnerability (Intra-plaque haemorrhage [IPH]; lipid-rich necrotic core [LRNC]; neovascularization; carotid plaque thickness; morphology and volume) whereas in the rows the 3 different types of technologies (CT, MRI at 3T and US). In the first column the white open arrow shows the intra-plaque haemorrhage detected with the 3 different technologies and the same is done for the LRNC in the second column. The neovascularization is showed in the column 3 and in the CT panel (top) the white open arrows show the pre and post-contrast phase demonstrating how the HU increase after administration of contrast material; similarly, in the MRI panel (medium), after contrast material the plaque (white open arrows) shows a significant increase of the signal intensity due to the enhancement of the plaque. In the panel of US the pre and post-microbubble injection show that in the plaque (white open arrow) there is significant enhancement due to the presence of microbubble into the plaque. In the fourth column the plaque thickness is showed; the white open arrows indicate the plaque whereas the red-dotted lines show the thickness of the plaque. In the fifth column a features of morphological vulnerability, the ulceration, is showed and the white open arrows shows the ulcer in CT, MRI and US. In particular, with US there are 2 panels showing the different sensitivity using conventional B-Mode with color-doppler and injection of micro-bubble: in this case the ulcer is visible with the only micro-bubble approach. The last column shows volume analysis and tissue segmentation in CT, MRI and US.

CEMRA= contrast enhanced Magnetic Resonance Angiography; CT = Computed Tomography; IPH= Intra-plaque Haemorrhage; LRNC = Lipid Rich Necrotic Core; MRI: Magnetic Resonance Imaging; US = Ultrasound

**Figure 2: Plaque reduction after statin therapy**

Plaque regression (reduction of lipid-rich necrotic core [LRNC]) in a 73 years old male patient before (July 2015, panel a-b-c) and after (July 2017, panel c-d-e) two years on statin therapy (Atorvastatin- dosage: 40 mg orally once a day) as seen on carotid plaque 3T MRI studies performed at different times. The Contrast-enhanced Magnetic Resonance Angiography (CEMRA) (panel a, white arrow) shows a significant degree of stenosis in the right internal carotid artery with a regression after 2 years (CEMRA, panel d, white open arrow). The basal axial T1-Turbo spin-echo with fat saturation (T1-TSE FAT-SAT) (panel b) shows large intermediate signal intensity plaque (white arrowhead). The axial T1 TSE FAT SAT acquired after 2 years (panel e,) shows a decreased plaque size (white open arrowhead). The basal axial T1 TSE FAT-SAT after gadolinium (panel c) shows enhancement of fibrous cap and adventitia with large lipid rich necrotic core (white curve arrow). The axial T1 TSE FAT-SAT post gadolinium acquired after 2 years (panel f) shows marked decrease of enhancement and a decrease of the LRNC covered by an intact fibrous cap (white curve open arrow). CEMRA= contrast enhanced Magnetic Resonance Angiography; FAT-SAT = fat saturation; LRNC = Lipid Rich Necrotic Core; TSE = Turbo Spin Eco.