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Carotid intraplaque haemorrhage: pathogenesis, histological classification, imaging methods and clinical value

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Abstract: Vulnerable carotid atherosclerotic plaques are characterised by several risk factors, such as inflammation, neovascularization and intraplaque haemorrhage (IPH). Vulnerable plaques can lead to ischemic events such as stroke. Many studies reported a relationship between IPH, plaque rupture, and ischemic stroke. Histology is the gold standard to evaluate IPH, but it required carotid endarterectomy (CEA) surgery to collect the tissue sample. In this context, several imaging methods can be used as a non-invasive way to evaluate plaque vulnerability and detect IPH. Most imaging studies showed that IPH is associated with plaque vulnerability and stroke, with magnetic resonance imaging (MRI) being the most sensitive and specific to detect IPH as a predictor of ischemic events. These conclusions are however still debated because of the limited number of patients included in these studies; further studies are required to better assess risks associated with different IPH stages. Moreover, IPH is implicated in plaque vulnerability with other risk factors which need to be considered to predict ischemic risk. In addition, MRI sequences standardization is required to compare results from different studies and agree on biomarkers that need to be considered to predict plaque rupture. In these circumstances, IPH detection by MRI could be an efficient clinical method to predict stroke. The goal of this review article is to first describe the pathophysiological process responsible for IPH, its histological detection in carotid plaques and its correlation with plaque rupture. The second part will discuss the benefits and limitations of imaging the carotid plaque, and finally the clinical interest of imaging IPH to predict plaque rupture, focusing on MRI-IPH.

Keywords: Carotid atherosclerosis; intraplaque haemorrhage (IPH); magnetic resonance imaging (MRI); stroke prevention

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Background

Stroke is the second most frequent cause of death in the world and the primary cause of long-term disabilities in western countries (1). At least 80% of all strokes are ischemic and 20% of them are the consequence of the rupture of a vulnerable carotid atherosclerotic plaque

located at the carotid bifurcation (1). As strokes resulting from carotid plaque rupture are known to be linked to embolism (2), plaque vulnerability assessment is likely an essential element in the detection of patients at risk for ischemic stroke and should be a factor when considering carotid endarterectomy (CEA) surgery (3). Currently, the

decision to perform CEA surgery is only based on the degree of carotid stenosis and cerebrovascular medical history (4). The link between stenosis degree and stroke risk has been widely demonstrated (5), and many national guidelines still claim that stenosis should remain the main criteria to classify plaques and thus the decision of CEA (6) even if evidence has been provided otherwise for asymptomatic patients (7,8) thus this sole factor may be insufficient to evaluate the risk-benefit ratio of CEA surgery in these patients (3,9). A meta-analysis showed that for most asymptomatic patients, a very few ischemic events happened after CEA, thus the risk-benefit ratio resulting from the CEA might be unfavourable (3,7,10). Moreover, the 10 years risk of stroke is only 4.6%, suggesting that 95% of the CEA performed on asymptomatic patient are unnecessary (11,12). Thus, it appears that management of CEA in asymptomatic patients need to be rethought and *in vivo* identification of vulnerable plaque has to be improved to be commonly used in a clinical setting for the decision of CEA surgery. In this context, non-invasive imaging methods and more specifically magnetic resonance imaging (MRI) appear to be the most sensitive and specific to identify IPH *in vivo* (13,14), especially in a clinical perspective to predict stroke risk (15-17). In this regards, ESVS guidelines encourage surgeons to use imaging to identify plaque with vulnerable factors in particular intraplaque haemorrhage (IPH) that needs CEA surgery (18). Several vulnerability factors were reported: thin fibrous cap, large lipid-necrotic core volume, monocytes infiltration (19) and IPH (19,20) resulting from immature neovascularization (21). It has been shown that all these factors are unequally involved in plaque destabilisation (19,22,23). Since 1936, IPH has been described as a risk factor of plaque rupture (24). During the 1980s several studies have demonstrated a relationship between carotid IPH and history of ischemic events (25,26). IPH is more prevalent in symptomatic stroke patients, regardless of the time since the event, than in asymptomatic patients (27-30). Carotid IPH has been documented by histological studies over the last 20 years, now it is likely an important factor to consider when classifying vulnerable plaques (26). Currently no drug treatment targeting specifically IPH is available; however statin use is associated with lower prevalence of plaque with neovascularisation (31) and IPH (32). On the contrary, platelet antiaggregant is associated with higher prevalence of IPH (32). In this context, *in vivo* detection of IPH appears to be one of the most reliable factors to predict cerebral ischemic events (15-17).

Carotid IPH

Pathophysiology

IPH is defined by the accumulation of blood components within the atheromatous plaque (26). IPHs are closely linked to the microcirculation within the plaque. McCarthy *et al.* showed that symptomatic plaques showed more, larger and more irregular neo-vessels than asymptomatic plaques (33). Indeed an increased neo-vessels density is associated with IPH and rupture of the plaque (33). In advanced atherosclerotic lesions, hypoxia, along with macrophages triggering inflammation and oxidative stress promoting low density lipoprotein (LDL) oxidation into oxidized LDL (Ox-LDL) processes are merged. All these factors lead to the chronic secretion of vascular endothelial growth factor (VEGF) increasing pathological impaired neoangiogenesis (19). Neo-vessels originating from the vasa vasorum develop through the medium and large arteries from adventitia to the intima (19). These neo-vessels, which lack smooth muscle cells and endothelial gap junctions, are disorganized and incomplete (21,34), and thus are prompt to leak. This results in IPH formation (20) and expansion (35) and the transfer of blood cells that promote plaque rupture (36). IPH carries inflammatory cells (37) that increase the necrotic core volume (38) also indirectly leading to vulnerable plaques rupture and subsequent clinic ischemic events (39).

During IPH, leucocytes, platelets and erythrocytes are released. The leaked erythrocytes break down into iron, cholesterol, glycophorin A and ceroids (24). The erythrocytes and leucocytes (37) extravasated from the lumen of neo-vessels into the atherosclerotic plaque, self-sustain inflammation and pro-oxidant mechanisms (19,40) (*Figure 1*). Indeed, in the hypoxic environment of the plaque, IPH-released neutrophils secrete angiogenic factors such as VEGF and lipid peroxidation by-products, (41,42) known risk-factors for future ischemic events (43). Neutrophils also abundantly express myeloperoxidase, which produce hypochlorous acid and H₂O₂, leading to a decrease in NO bioavailability thus enhancing endothelial dysfunction (44,45). Ultimately, the lysis of neutrophils release highly pro-oxidant materials, such as DNA histones (46). Moreover, the activation of NAD(P)H oxidases and myeloperoxidases in macrophages are known to produce superoxide (O₂^{•-}) enhancing a pro-oxidant environment. To reduce IPH, the leaked erythrocytes are phagocytosed mainly by macrophages. This results in

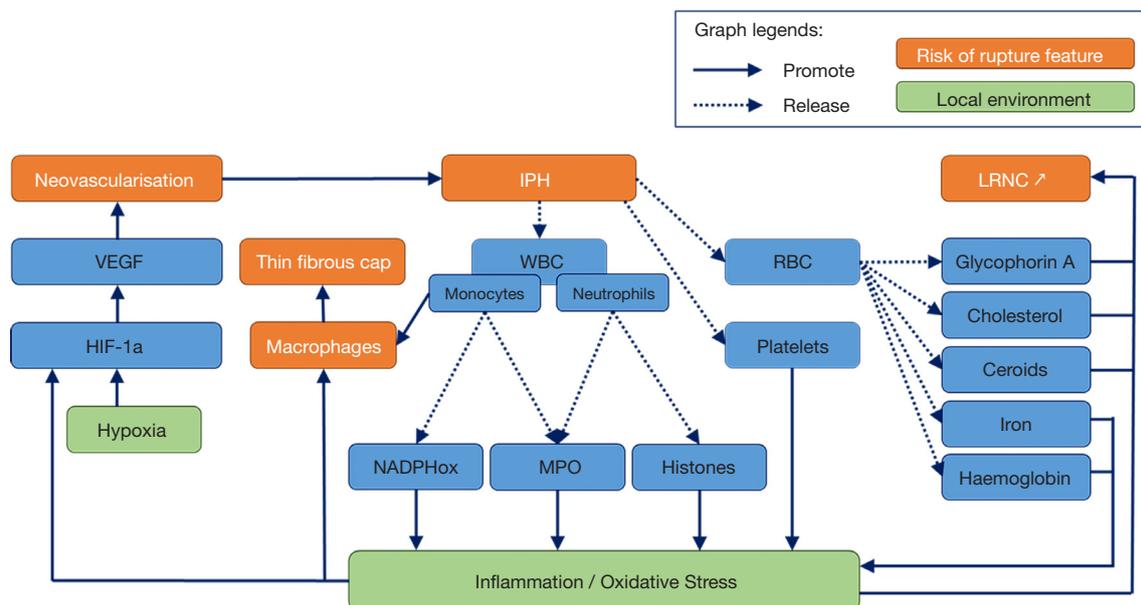


Figure 1 Inflammation and oxidative implications of IPH. IPH, intraplaque haemorrhage; LRNC, lipid rich necrotic core; MPO, myeloperoxidase; NADPHox, nicotinamide adenine dinucleotide phosphate oxidase; RBC, red blood cell; VEGF, vascular endothelial growth factor; WBC, white blood cell.

the release of haemoglobin and iron which are highly pro-oxidant (24) and pro-inflammatory through the conversion of H_2O_2 into the highly toxic hydroxyl (OH^\bullet). In addition, the erythrocytes plasma membrane, which is composed of 40 percent cholesterol, is the primary source of necrotic core expansion during phagocytosis (20,24,47). Therefore regulation mechanisms are implemented to reduce IPH, such as the anti-inflammatory, cytoprotective shift in macrophages phenotype and the recruitment of haptoglobin that metabolise haemoglobin and recycle iron (48). Unfortunately, they are quickly depleted and become inefficient. Consequently, these closely integrated pro-inflammatory and pro-oxidant processes persist which not only enhances IPH but also promotes the growth of the necrotic core (19,49), increasing the risk of ischemic event (36).

Typically the presence of blood in the atherosclerotic plaque is due to leaky neovessels localized to the plaque shoulder (50). However, in different areas, other mechanisms can lead to the presence of blood within the plaque. Cholesterol crystals due to eryptosis can mechanically break neovessels, which bleed into the atheromatous plaque (51,52). Moreover, cholesterol crystal content are independent predictors of thrombus and cardiovascular events (52). Intraplaque blood can also originate from the integration of a luminal erythrocyte-

rich thrombus with the plaque (24,53) or entry of luminal blood (20). Plaque fissures are observed even in plaques with an intact fibrous cap and those that are co-localised with fresh IPH (54). In this case, the thrombus is entrapped into extracellular matrix leading to a narrow lumen, yet it also appears to be a healing process. Histologically, this healing process can be hard to discriminate from IPH (24).

Histology

IPH determination by histology

Histology is the gold standard in studying plaque components like IPH, lipid rich necrotic core (LRNC) and inflammation. Usually, the surgically removed atheromatous plaque also includes the middle part of the media and internal part of the media. In clinical context, surgical pathologists generally evaluate IPH on plaque slices after hematoxylin-eosin (H&E) or Masson trichrome stains, which are non-specific staining for haemorrhage (25,55).

To improve the histological IPHs identifications, several biomarkers are used. The most frequently used is iron, as it links to haemoglobin and is released during erythrocyte phagocytosis; it is highlighted in the adventitia by the Perls technique with Prussian blue stain (Figure 1). Red blood cell (RBC) membrane specific protein cholesterol

Table 1 Histological classifications to determine plaque vulnerability

Classification	Reference	Application	Goal	Features	Stages
American Heart Association classification	Stary 1994; Stary 1995 (62,63)	Histology	Graduate atherosclerosis severity	Isolated macrophages foam cells	Type 1 (initial) lesion
				Mainly intracellular lipid accumulation	Type 2 (fatty streak) lesion
				Type 2 changes & small extracellular lipid pools	Type 3 (intermediate) lesion
				Type 2 changes & core of extracellular lipid	Type 4 (atheroma) lesion
				Lipid core & fibrotic layer, or multiple lipid cores & fibrotic layers or mainly calcific or mainly fibrotic	Type 5 (fibroatheroma) lesion
				Surface defect, hematoma-haemorrhage, thrombus	Type 6 (complicated) lesion
Lovett classification	Lovett 2004 (64)	Histology	Graduate atherosclerosis severity	Definitely stable, e.g., predominantly fibrous, few inflammatory cells, intact cap	Grade 1
				Probably stable, e.g., one feature of instability such as small haemorrhage or inflamed	Grade 2
				Probably unstable, e.g., inflammation, thin cap, and large core but no rupture	Grade 3
				Definitely unstable, e.g., rupture, thrombus, large haemorrhage, thin inflamed cap	Grade 4

crystals are detected by immunohistochemistry with glycophorin A, allowing the discrimination of lipid-rich and erythrocyte-rich parts of the necrotic core (*Figure 1*). Ceroids (56,57) are often co-localised with haem (24) and are markers of senescent RBC but are less specific of IPH than cholesterol crystals (24). They are identified by Raman or fluorescence spectroscopy and are completed by other peroxidation markers. Neutrophils markers such as matrix metalloproteinase-9 (MMP-9), neutrophil gelatinase-associated lipocalin/MMP-9 (NGAL/MMP-9), elastase, CD66b, proteinase 3, myeloperoxidase (MPO) or α -defensins are colocalised with IPH (58). Neutrophils are predictors of recurrent ischemic events.

As these markers are specific to different forms and localisations of RBC, multiple consecutive slice staining could elucidate RBC trafficking and IPH. Moreover, in vulnerable plaques, neo-angiogenesis-derived IPH could be difficult to discriminate from integrate coagulum which is a sign of plaque regression (24). CD34 immunostaining allows for a precise quantification of the micro-vessels density (59). Plaque neo-vascularisation can also be visualised by H&E

staining of endothelial cells (60) or von Willebrand factor (factor VIII) staining (61). To characterise leaky neo-vessels, SMC should also be stained for smooth muscle antibody (SMA), as the presence of factor VIII and absence of SMA indicates that the vessel is leaky.

IPH and vulnerable carotid atherosclerotic plaques in histology

Vulnerable carotid atherosclerotic plaques are composed of various components (*Figure 1*). The lipid-rich necrotic core harbours lipid content such as cholesterol crystals, but also calcifications and haemorrhagic components. As vulnerable plaques continually evolve, different classifications have been established (*Table 1*) (62-64) in order to stratify the associated plaque instability and thus the subsequent ischemic risk.

After surgery, histopathological classifications assess the vulnerability of the carotid atherosclerotic plaque. The American Heart Association classification was the first that aimed to graduate plaque vulnerability (62,63), and more recently, Lovett proposed his own classification, adding

Table 2 IPH stages according to Derksen's classification (70)

IPH stages	Characteristics	IPH age
Recent IPH	Contains unorganised fibrin, intact and some debris of erythrocytes	<2 weeks
Organised IPH	Contains an increased concentration of fibrin, some peripheral capillary and smooth muscle cells as well as a mixture of intact and debris of RBC	Between 2 and 6 weeks
Amorphous IPH	Characterized by disorganized materials and a lack of well delimited cells	>6 weeks
Amorphous IPH with calcifications	Characterized by disorganized materials and a lack of well delimited cells and calcifications	>6 weeks

IPH, intraplaque haemorrhage; RBC, red blood cell.

the inflammation item (64). IPH is an evolving process due to the different pathophysiological processes that are chronologically involved and the subsequent progression of ischemic risk.

In the preclinical setting, mice (ApoE^{-/-} and LDLR^{-/-} under high cholesterol diet) are the most commonly used animals to study atherosclerosis. However, they are poor experimental models of IPH, neovascularisation and plaque rupture. Therefore, IPH data essentially originate from human clinical studies (24) or from other animal species that develop vulnerable carotid atherosclerotic plaques similar to human plaques. For example, annexin V (which is highly released during eryptosis) injection in aortic atheromatous plaque of de-endothelialized rabbits under cholesterol diet leads to the necrotic core growth, free cholesterol crystals formation and higher macrophage recruitment (65) suggesting the role of eryptosis in unstable atherosclerotic plaques. This suggests that in addition to a direct increase in plaque rupture risk (15), IPH might participate in a pathophysiological vicious circle leading to plaque rupture.

Analysing the plaque components, IPH presence is generally associated with a thin fibrous cap and a large necrotic core (19). Pelisek *et al.* found in symptomatic and asymptomatic patients that neo-vessels were present in 93.8% of the plaques with over 70% stenosis and 97.1% of these neo-vessels were immature and leaky leading to IPH (66). A histological study (n=526) led by Redgrave *et al.* on patients with symptomatic carotid plaques who underwent CEA reported that 64.6% of the plaques showed IPH (67). This study also demonstrated that IPH was associated with fibrous cap rupture, independent of other risk factors [OR =3.00 (1.64–5.51)] suggesting that IPH is a contributing factor to plaque vulnerability (67). Vrijenhoek's team reported that IPH was more frequent in men (67%) than in same aged post-menopausal women (54%), while micro-vessels density was not significantly different (68).

Moreover, in the same study IPH leads more often to plaque rupture in men than in women [HR =1.5 (1.1; 2.1)] (68). In most cases, IPH presence was associated with other known risks factors of rupture: necrotic core expansion (19), leaky neo-vessels (20), macrophages accumulation and oxidative stress (19,26).

An Anglo-Dutch group published a histochemistry analysis on symptomatic patients with moderate stenosis. They studied the link between risk factors traditionally associated with plaque rupture and cerebral cardiovascular outcomes (69). Statistical analysis of 1,087 carotid plaques revealed that macrophages infiltration, thrombosis and micro-vessels density [OR (micro-vessels density) 1.49 (1.05; 2.11)] was significantly correlated with plaque vulnerability. On the contrary, no relationship was observed between plaque vulnerability and fibrous cap, lymphocyte infiltration or IPH [OR (IPH) 1.15 (0.84; 1.59)]. The authors suggested that the contradictory findings of neovascularization and IPH could be explained by a blood leakage only present during early IPH in organised stages (*Table 2*). Nevertheless, this hypothesis was not experimentally tested. Another explanation could be that IPH and thrombosis are hardly discriminable.

IPH histological classifications

None of the previous classification of vulnerable atherosclerotic plaques (62-64) take into account the evolution of IPH, thus several IPH histological classifications were established (70-72). These classifications (71,72) are not commonly used anymore because the classification of Derksen is the most complete and is currently used in anatomopathology. This classification distinguishes four stages of IPH (*Table 2*) based on histological H&E stain.

According to Derksen's histological classification, organised IPHs are more vulnerable to breakdown than

amorphous and recent IPHs, mainly due to macrophages presence in organised IPHs zones (70). Derksen *et al.* reported that 81% (over 794 studied) of carotid plaques showed an IPH spreading over an average of 5% of the total plaque volume (70). Among these IPHs, 2% were recent, 11% organised, 75% amorphous, and 9% amorphous with calcifications.

However, so far there is no method that can allow *in vivo* diagnosis or follow-up (i.e., imaging) of IPH stages since it is currently only assessable after CEA by histology. Moreover, this criterion is currently not suitable for the clinical decision, because plaque rupture is multifactorial (19,73,74) and other biomarkers need to be taken into account to stratify the ischemic risk (16).

All plaque risk factor of rupture should be evaluated in order to predict future ischemic event, but particular attention should be given in IPH stages assessment especially in the CEA decision for asymptomatic patients.

Imaging

Over the last several years, several imaging techniques have been developed to reliably analyse carotid atherosclerotic plaques composition. In a clinical context, Doppler ultrasonography (75) and computed tomography (CT) (9,10,76) of the supra aortic trunks are commonly used to accurately assess the degree of stenosis. Magnetic resonance angiography (MRA) is being increasingly used (13,77) because it is non-radiant, less nephrotoxic compared to CT, and more accurately examines the cerebral parenchyma.

Ultrasound and contrast enhanced ultrasound

Ultrasounds (US) are the best method to assess the carotid stenosis by imaging (75) but is highly operator dependent. They allow to study the morphologic and hemodynamic features of the plaque by assessing tissues echogenicity. Without contrast medium IPH is hard to discriminate from LRNC only based on carotid plaque echogenicity (78,79). Contrast enhanced US (CEUS) are able to assess neovascularisation *in vivo* in real time (80). IPH can be indirectly visualised in ultrasonography through the vasa vasorum and neovessels analysis by CEUS (80-82); if results of the sole US examination are inconclusive, presence of neovessels observed with CEUS in the carotid plaque might underline IPH presence. Contrast enhanced microbubbles allow for the visualization of vascularized lesions in hyper echogenicity (82). Results on carotid ultrasound with

contrast agent attest that an increased vasa vasorum (83) and microvessels (80) density can enhance the IPH risk. Most of the plaques harbouring an heterogeneous pattern (mixed echoes and anechoic areas) presented IPH at the histological analysis (84).

CT and positron emission tomography (PET) scans

CT is applied in a clinical setting to diagnose morphological abnormalities as stenosis (85), aneurysm or carotid dissection (86) through the tissues density analysis. Computed tomography angiography (CTA) with iodinated contrast medium is required to analyse carotid arteries, but it is also known to underestimate the degree of stenosis (87). However, studies suggest that CTA is able to discriminate IPH parts from lipid-rich and fibrous parts in carotid atherosclerotic plaques (88,89) even if the densities are almost similar (90). Indeed, according to a recent study, IPH can be detected with high sensitivity and specificity by CTA according to attenuation at 25 Hounsfield units (HU) (88). Moreover, calcified rim and soft internal plaques indirectly predict IPH actual presence (91). On symptomatic patients, intraluminal thrombus can be visualized (92). Thus, CT scan is a very specific non-invasive method, but with a limited sensitivity (87). U-King-Im *et al.* compared CT scans and MRI techniques and found the MRI is a better tool to visualise IPH through plaque ulceration (89). Moreover, CT exposes patients to radiation and thus it is not the first-choice technique to assess IPH.

PET scan is an imaging technique with a high sensitivity, but also exposes patients to radiation (93). PET scan is growing imaging technique that is able to measure metabolic activity in different parts of the body. It allows for the visualisation of angiogenesis and macrophage infiltration, and can discriminate lipid-rich plaque from fibrous plaque (94) but failed so far to identify IPH. Several contrast agents have been developed, but fluorodeoxyglucose (FDG) contrast agents targeting macrophages appear to be the most used; they detect inflammation and present good association with histology on carotid and aorta (94) giving details on plaque metabolism. It remains to be demonstrated that the PET signal can reliably predict future long-term cardiovascular events on carotid imaging. With an FDG contrast agent, it is possible to visualise neovessels into the intima (95). Moreover, ¹⁸F-FDG-PET allows the precise visualisation of the plaque anatomy confirmed by guided MRI (96,97). Fluorine F 18-sodium fluoride (¹⁸F-NaF) is a radioactive tracer that can be used in PET-

MRI, it could be a useful imaging technique to characterise vulnerable plaque; tissue sections with high ^{18}F -NaF uptake demonstrated calcification, macrophage infiltration and cell death (98). New contrast agents are needed to precisely characterise IPH.

MRI

The MRI-IPH imaging is a direct method based on iron detection with T1 weighted sequences that produces an intraplaque hyperintense signal (99,100), while other carotid components are visualised as isointense or hypointense signals (101,102). The first sequence validated by histology was a T1-weighted sequence called Magnetic Resonance Direct Thrombus Imaging which is able to detect methemoglobin (103). This sequence detects methaemoglobin-rich haemorrhages, with a strong relationship to histologically confirmed complicated plaques (103). Other sequences were developed, such as 3D Magnetization-Prepared Rapid Acquisition Gradient-Echo (104), three-dimensional T1-weighted Turbo-Spin-Echo sequence (105), Simultaneous Non-contrast Angiography and intraPlaque haemorrhage (106), T1 weigh inversion recovery 3D fast field echo sequence (107) or 3D Spoiled gradient recalled echo pulse sequence for Hemorrhage assessment using INversion recovery and multiple Echoes (3D SHINE) sequences (108). The “black blood” sequence needs no contrast agent to discriminate a LRNC from a lipid core with IPH, as well as other plaque components with a good correlation to histology (109,110). These sequences are specific and sensitive to detect IPH, but they failed in the identification of IPH stages (70). Further sequences such as “black blood” and “gradient echo”, “spin echo” or “fast spin echo” sequences are currently investigated to identify IPH (107).

MRI sensitivity can be enhanced with a dynamic contrast agent, via an intravenous bolus (111). Contrast enhanced MR angiography (CE-MRA) shows a higher sensitivity, specificity, positive predictive value, negative predictive value with a shorter acquisition time and less artefacts for IPH detection (100). Gadolinium enhancement is used to visualise fibrous cap integrity, plaque neovascularization (112), and inflammatory infiltration (113) it also helps to confirm IPH visualised in T1 sequences (112,113). Dynamic contrast intensification techniques can better visualise neovascularisation and inflammation (114). New sequences are currently being investigated in order to better characterize IPH dating and at the same time identify

other vulnerable plaque features, in order to be used in a clinical setting.

Several studies showed a relationship between histological IPH presence and different stages of IPH assessed by MRI (77,115). Depending on classifications, a T1 hyperintense signal is observed if the IPH is fresh, but this hypersignal weakens over time. Limits of this classification may be correlated to the signal intensity variation, because of the interpersonal chemical composition variation in the plaque. Indeed, throughout the brain T1-weighted sequence application, the haemorrhage signal can evolve from hyperintense to hypointense because of the transformation of methemoglobin into hemosiderin (109). Contrary to this, carotid IPH signal can remain hyperintense for more than 18 months (116). The variability of the signal intensity may help in the detection of plaques involved in ischemic events (117) and can also coincide with the different IPH stages, particularly between amorphous and organised stages (70) according to Derksen’s classification (70). Nevertheless, this hypothesis is still not validated by any study. Methemoglobin signal can mix with other tissues signals, such as calcified tissue or hemosiderin, leading to false negatives (100). On the contrary, perivascular-derived signals of adipocytes can lead to IPH false-positives (100). In order to accurately imaging carotid plaque morphology, histopathological variability should be taken into account (118). Indeed, IPH dating by MRI needs to be improved and sequences need to be standardised before clinical use (24,26).

IPH and clinical outcomes

IPH evaluated by histology and clinical outcomes

Although IPH consideration is increasing in the clinical evaluation of the plaque, it should be analysed together with other interrelated clinical and biological factors (i.e., Inflammation, neovascularisation, fibrous cap thickness, necrotic core volume and composition).

Redgrave *et al.* demonstrated on 526 symptomatic patients that IPH was associated with a previous stroke, transient ischemic attack (TIA) or amaurosis fugax (67). Another study showed that IPH is associated with cerebrovascular events risk (68) but only in men, which is a well-known cardiovascular risk factor. Few histological studies demonstrate the importance of identifying carotid IPHs on stroke prognosis. A significant study (n=818) by Hellings *et al.* on symptomatic and asymptomatic stroke

patients who underwent CEA (25) found that IPH was present in 69.9% of asymptomatic plaques and 76.3% of symptomatic plaques. Moreover, IPH increases the risk of cardiovascular events from 17.2% to 30.6% on a 3-year period [HR =1.7 (1.2; 2.5)] independent of perioperative events. IPH were also associated with primary outcomes (vascular death, nonfatal stroke, non-fatal myocardial infarction and vascular intervention), stroke and non-stroke vascular events, underlying an overall risk of cardio-vascular events. Other plaque features were not associated with any cardiovascular event thus IPH presence could improve global health care.

Consequently, imaging methods which are able to visualise neo-vessels and IPH should lead to a better understanding of the vulnerable plaque evolution. The challenge of current research is to be able to diagnose *in vivo* IPH that may cause ischemic events before they happened, in order to improve CEA decision.

Correlation between MRI assessed IPH and clinical events

As previously stated, IPH is an evolving process. The different stages of IPH are unequally involved in plaque vulnerability affecting the subsequent ischemic risk (70). Currently, no imaging technique is precise enough to discriminate Derksen's IPH stages. As blood components break down over time, they have a different chemical composition and magnetic properties (109) thus MRI might be able to identify Derksen's IPH stages (70). However, IPH assessed by MRI (MRI-IPH) appears to be a promising tool to predict cerebral ischemic events as stroke, TIA or amaurosis fugax.

Retrospective studies

Several studies have shown that MRI-IPH is an important tool to assess the plaque risk of rupture in symptomatic patients (119). Retrospective studies have established a positive relationship between unstable plaques detected by MRI and the latest neurological symptoms (120). MRI-IPH was also correlated with prior cardiovascular events (29) independent of stenosis degree, age, sex, hypertension, and smoking habit (107). Singh *et al.* [2013] performed a multivariable analysis and demonstrated that MRI-IPH was associated with the composite cardiovascular event (i.e. angioplasty, stenting or bypass graft) (OR =3.26; 1.14–9.37, P=0.028) (107). A recent study suggests that IPH detected by MPRAGE is a strong indicator of acute focal cerebral infarction (121), moreover IPH increased the risk of acute

cerebral ischemic event from 22% to 47% (122). In another study, recent MRI-IPH was associated with ipsilateral stroke and TIA but only for symptomatic patients. This same study concluded that other risks of plaque rupture might be taken into account to predict ipsilateral stroke (15), while another study showed that IPH was associated with stroke (123). Symptomatic patients had a higher prevalence of cerebrovascular events recurrence (107) and an increased risk of subsequent cardiovascular event (117,119). In two studies, all patients with an MRI T1 hyperintense signal had a greater recurrence risk for ischemic ipsilateral events (117,124), as IPH is a risk factor for further carotid IPH (116).

Prospective studies

As some links between cardiovascular, cerebrovascular and IPH were suggested by retrospective studies (29), prospective studies were conducted. MRI appears to be an interesting tool to assess IPH in asymptomatic patients (16). According to Saam *et al.*, the presence of IPH increases the risk of cerebrovascular events 5.69 times, with an annualised event rate of 17.7 % with IPH and 2.4% without IPH (99). Moreover, carotid plaques containing IPH and a ruptured fibrous cap are highly prone to develop ischemic events (99). Thus, this study showed that IPH diagnosed by MRI is a better predictor of stroke than stenosis (99). Other studies showed that IPH, in association with other rupture risk factors (thin fibrous cap, lipid-rich necrotic core), predicts subsequent cerebrovascular events (16) and ischemic events (stroke or TIA) (101). Symptomatic and asymptomatic patients (101,116,125,126) with carotid MRI-IPH, had an increased risk of subsequent cardiovascular events.

As MRI-IPH appears to predict future cerebrovascular ischemic events (68) and is highly discriminative from other plaque components such as necrotic core, macrophages inflammation and fibrous cap (115,127), it could become a valuable target to identify vulnerable carotid atherosclerotic plaques, but it is necessary to standardise the sequences and validate this technique in both genders in order to establish a strong correlation for all populations (68).

Conclusions

Embolism of vulnerable carotid atherosclerotic plaques is a frequent cause of ischemic stroke. Prevention for carotid plaques to become vulnerable is challenging. *In vivo* tools to assess reliably the factors of carotid plaque evolution are required. It has been demonstrated that IPH is a good predictor of plaque vulnerability and stroke incidence.

Several techniques have been developed to assess IPH such as Doppler ultrasonography and CT. Nevertheless, MRI appears to be the most effective way to assess IPH *in vivo* with reduced risk for the patient. Although MRI may currently be the best way to determine IPH stage, new sequences are required to improve its sensitivity before implementation in clinical practice. Reducing the examination duration, increasing the specificity of the diagnostic alone or in association with other MRI markers of plaque vulnerability could be additional challenges. It also might be relevant to compare *in vivo* imaging analysis to serum biomarkers (128) to better assess vulnerability of the carotid atherosclerotic plaque. A large-scale cohort study is required to validate the MRI sequences used to diagnose *in vivo* IPH as a predictor of cerebral ischemic events.

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