Imaging non-coronary atherosclerosis: a model to understand coronary artery disease in humans

Augusto Gallino, Alessandra Pia Porretta, Nicola Bianda, Marcello Di Valentino, Julija Klimusina, Paolo Santini, Costanzo Limoni, Jeanne Marie Segatto, Isabella Sudano, Roberto Corti, Rolf Wyttenbach

An interdisciplinary Report from the Ospedale San Giovanni Cardiovascular Imaging Group in Bellinzona, Switzerland

Summary

While we know a great deal about molecular mechanisms of plaque evolution, investigated in atherosclerotic animals, the transition from plaque stability to instability has been hampered by a lack of experimental models that faithfully reflect human disease. The abrupt clinical presentation of acute coronary events strongly indicates discontinuity in the natural history of atherothrombosis. The causes of such discontinuity are complex and probably multiple. Invasive cardiology has allowed us to understand several mechanisms of disease *in vivo* confirming many data and hypotheses formulated by pathologists in the past. The intrinsic limitation of invasive cardiology is however its invasiveness. Non invasive study of plaque morphology of coronary atherosclerosis is still in its infancy given the big challenge to obviate respiratory and cardiac movements which hamper delineation of details of plaque morphology. In this article we report the clinical research experience at our research cardiovascular unit in Bellinzona in the field of *in vivo* imaging of non-coronary atherosclerosis taking advantage of the fact that non-coronary atherosclerosis shares many facets of coronary artery disease.

Key words: atherosclerosis; vascular imaging

Atherosclerosis and the role of vascular imaging

Atherosclerosis is a systemic and multifocal disease, which starts early in life and usually takes decades before overt disease eventually appears as a consequence of progressive obstruction or abrupt thrombotic occlusion. This silent course makes it necessary to develop predictors of disease long before symptomatic lesions develop [1]. In addition to several classical risk factors and new emerging humoral risk predictors, imaging may constitute a formidable diagnostic and prognostic tool in order to identify presence, extension, progression (or regression) of disease as well as vulnerability of the atherosclerotic lesions [1]. Vascular imaging can potentially offer a comprehensive assessment of atherothrombosis even at the preclinical stage, thus improving primary prevention, assessment of the burden, extent and progression/or regression of atherosclerosis. It may also be helpful in defining stable versus unstable atherosclerotic lesions in improvement of secondary prevention and treatment, and in understanding mechanisms of disease.

Non-coronary arteries: a human model to evaluate atherosclerosis

Historically non-coronary arteries have been a model for the development of new diagnostic and therapeutic modalities (B mode ultrasound, Doppler flow velocity measurements, angiography, bypass surgery, thrombolysis, percutaneous procedures) which were later applied to the coronary field. A paradigm example is angioplasty, which was initially designed to treat atherosclerotic obstructions of the lower limb arteries [1, 2] and which allowed Andreas Grüntzig, who started his experience in the field of extra cardiac vascular medicine, to apply this technique to the coronary circulation [3]. A further example of how peripheral vascular medicine may be relevant to explore new cardiovascular revascularisation strategies is angiogenesis. Intensive research in this field, as has been performed by J. Isner and others, allowed new insight into the complex issue of critical limb ischaemia, microcirculation and potential treatment modalities which may be relevant for other territories (i.e., myocardium) [1, 4]. Non-coronary arteries may be considered a "window" for the insight into pathophysiology, epidemiology, and prognosis of cardiovascular disease (brachial artery flow-mediated dilation, intima-media thickening and plaque imaging of the carotid arteries). We have focused our attention during the last 15 years on imaging of atherosclerotic manifestation at the level of the ca-

Funding / potential competing interests:
AG is recipient of a grant from the Swiss Heart Foundation. RC is recipient of a grant from the Swiss National Research Foundation (SNF 320000-109905).

Correspondence:
Professor Augusto Gallino, MD
Cardiovascular Research Unit
Ospedale San Giovanni (EOC)
CH-6500 Bellinzona
Switzerland
agallino[at]bluewin.ch

rotid arteries, thoracic aorta and lower limb arteries. We have explored distinct imaging techniques such as Multi detector computer tomography (MDCT) [5] for carotid arteries and later on MRI using high resolution Plaque MRI. The latter allowed us to firstly describe the mechanisms of angioplasty *in vivo* and non-invasively, and the complex process of re-stenosis (fig. 1) [6, 7]. This allowed us to exploit the same technique (plaque MRI) to also investigate the natural process of plaque progression *in vivo* in a prospective cohort study, and to delineate the natural history of atherosclerosis at the level of carotid and peripheral arteries [8]. The results of this study, supported by a grant of the Swiss Heart Foundation (2005) and the Swiss National Science Foundation (SNF), showed the interesting finding of distinct intra-individual remodelling pattern in two distinct vascular territories during a 2 year follow-up period (fig. 2). The results of this study were determinant in generating a running project aiming at identifying various mechanisms of progression of atherosclerosis, i.e., to verify *in vivo* the hypothesis of the role of plaque inflammation and plaque neovascularisation on progression and vulnerability of atherosclerosis when compared with distinct remodelling process of the vessel wall.

Emerging concepts for the understanding of the atherosclerotic process

Plaque composition and plaque remodelling

During a life-time, most atherosclerotic plaques remain asymptomatic, some may become obstructive, and a few may become vulnerable by rupture, erosion, or thrombosis and can lead to atherothrombotic events such as acute coronary syndromes, stroke, and critical limb ischaemia [1, 9]. It is in the field of plaque composition and vulnerability that pathologists have greatly contributed to the understanding of atherosclerosis and of its complications [1, 10–12]. Ruptured and rupture-prone plaques contain a large and soft lipid-rich necrotic core covered by a thin and inflamed fibrous cap, the so-called thin cap fibroatheroma (TCFA) [13]. It is the merit of the pathologist S. Glagov who identified, more than 20 years ago, the role of expansive arterial remodelling as a major determinant of the extent of luminal narrowing in all vascular beds, a deterministic mechanism for maintaining patency of lumen in the presence of atherosclerosis [14]. Conversely, there is some evidence that arteries undergoing important positive remodelling may harbour a high risk of complications and are more prone to plaque complications, such as plaque rupture with exposure of the lipid rich core or plaque haemorrhage [10–12].

Figure 1

A–D Axial, proton-density weighted high-resolution MR images of the right leg at the level of the superficial femoral artery, shows chronic high-grade pre-occlusive stenosis (or partially recanalised chronic occlusion) pre-PTA (A, detail C) and post-PTA (B, detail D). The residual lumen is highlighted in red in the details pictures (C and D). The arrows mimic the projection normally used in X-ray diagnostic. MRI provides *in vivo* evidence of extensive plaque disruption induced by balloon angioplasty, and may explain mechanisms of complications of this technique. In addition, it highlights the potential overestimation of PTA results by X-ray angiography as it underestimates the residual plaque size.

E–F Angiography pre- (E) and post-PTA (F) of the right superficial femoral artery with a successful post-PTA angiographic result.

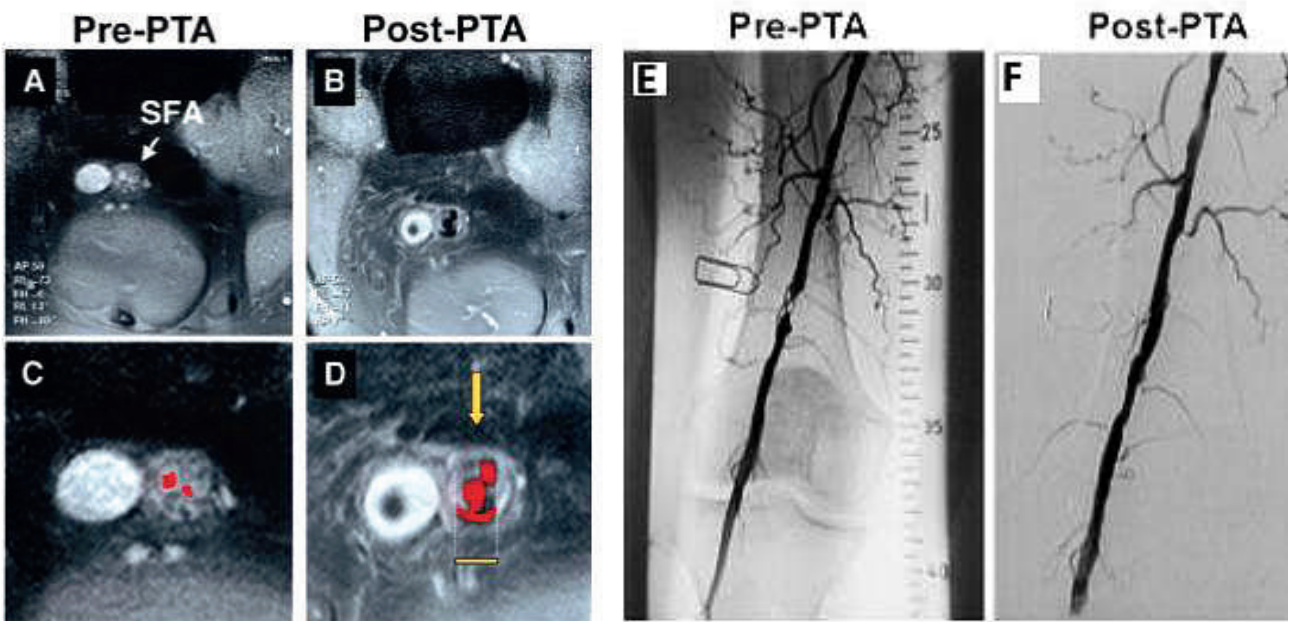
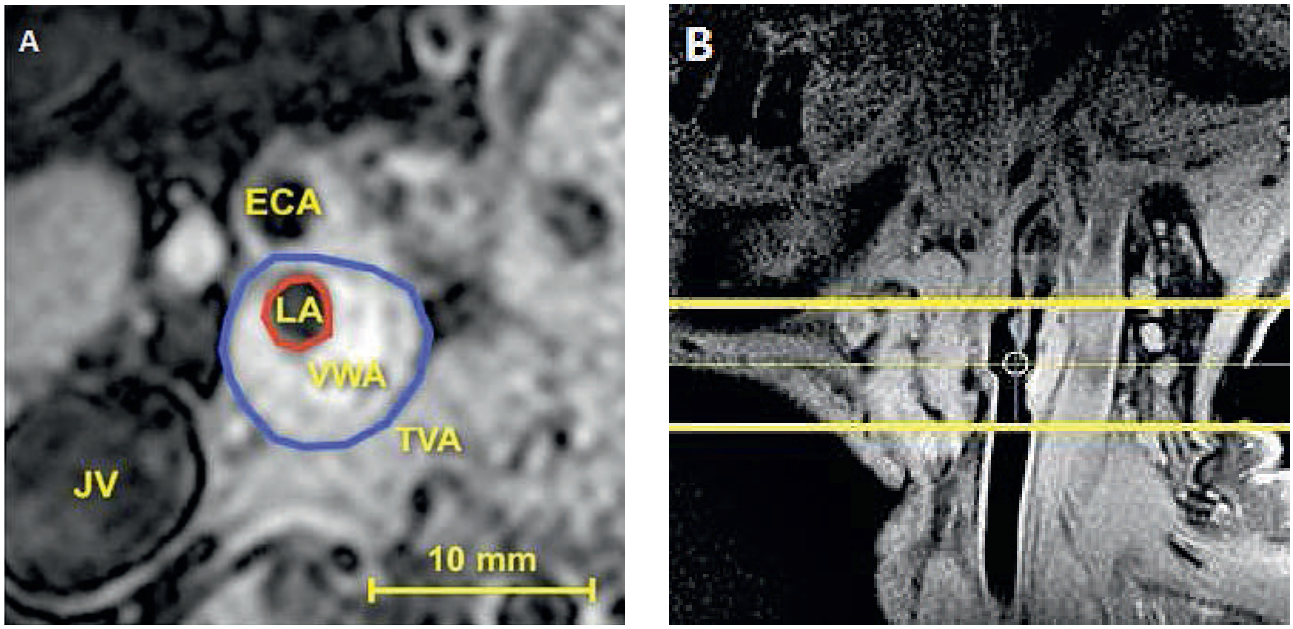


Figure 2

A The figure shows the corresponding atheromatous axial MRI image and indicates important excentric vessel remodelling. LA = lumen area; TVA = total vessel area; VWA is calculated by substracting LA from TVA (VWA = TVA–LA); JV = jugular vein; ECA = external carotid artery.

B Sagittal high-resolution MRI (hr-MRI) image at the level of the carotid bifurcation shows an extensive atherosclerotic plaque with positive remodelling of the internal and common carotid artery (T1w, double inversion recovery). The yellow bars indicate the area where axial hr-MRI sequences are acquired.



Inflammation

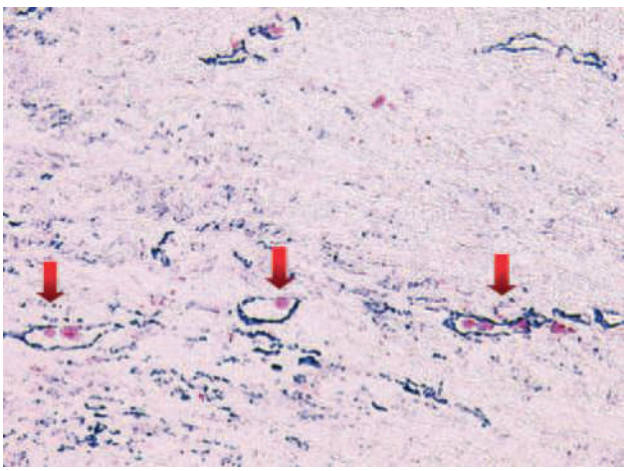
Atherosclerosis was considered to be a chronic lipid storage disorder until 40 years ago. Today, there is overwhelming sound scientific data demonstrating that this disease involves an ongoing inflammatory response in all stages of disease from the beginning through progression and finally during late atherothrombotic complications causing acute cardiovascular events. Atherosclerosis is a systemic, mainly lipid-driven inflammatory disease of the arterial wall leading to multifocal plaque development [1, 9], as elegantly demonstrated in a recent study on cerebrovascular ischaemic disease [15]. Animal experiments have clearly shown how the vascular cell adhesion molecule-1 (VCAM-1) exactly binds the types of leukocytes found in early experimental atherosclerotic plaques (i.e., the monocyte and T lymphocyte) [1, 16]. Many interactions between inflammatory response and the complex process of atherosclerosis have been elucidated including the interaction with both lipid and NO metabolism. One recent interesting relationship observed during initial and advanced manifestations of atherosclerosis is the one between adventitial and plaque neovascularisation and inflammatory cells (fig. 3).

Plaque-neovascularisation and plaque-haemorrhage

Vasa vasorum are present in the adventitia of the arterial vessels and nourish the external components of the vessel wall, while the intima is normally fed by oxygen

Figure 3

Histological evidence for atherosclerotic neovascularisation as a pathway for macrophage infiltration in human aortic plaques obtained at autopsy. Bicolour, contrasting immunohistochemical technique showing micro-vessels in cross sections identified with the monoclonal endothelial cell marker CD34 linked to a blue chromogen and inflammatory cells identified with a combined macrophage/T cell marker CD68-CD3 linked to a red chromogen. (Courtesy Prof. K. Raman Purushothaman)



diffusion from the lumen of the artery [17]. Animal experiments have shown how, as disease progresses, the intima becomes thickened and oxygen diffusion is hampered. These vessels (vasa vasorum) generally originating from bifurcation sites of epicardial coronary vessels or aortic arch branches behave functionally, at least in part, as normal arteries (i.e., by vasoconstriction or vasodilatation) according to various stimuli apart from reaction to norepinephrine and angiotensin II probably in order to protect the vessel wall from is-chaemia during protracted sympathetic activity [1, 17]. The molecular basis of angiogenesis is not completely elucidated but probably involves hypoxia as a major stimulus. The sprouting of the new capillaries and new vessels is mediated by progenitor and/or endothelial cells [17]. Experimental animal studies have shown that neo-vascularisation accompanies the process of wall re-modelling, where the latter is frequently associated with higher risk lesions. Interestingly enough, neovascularisation has been observed in early and late phases of atherosclerosis concomitantly with inflammatory infiltrates and accumulation of apoliprotein A-I [17]. Vasa vasorum play an important role in recruitment in high-risk plaque including the shoulder and the cap of the plaque. A recent study confirmed higher co-pre-valence of vascular cell adhesion molecules around neo-vessels in human tissue when compared to arterial endothelium. Neovessels content was significantly increased in plaques with moderate to severe inflammation (fig. 3) [17].

Emerging non invasive tools for the evaluation of atherosclerosis in humans

Contrast-enhanced ultrasound

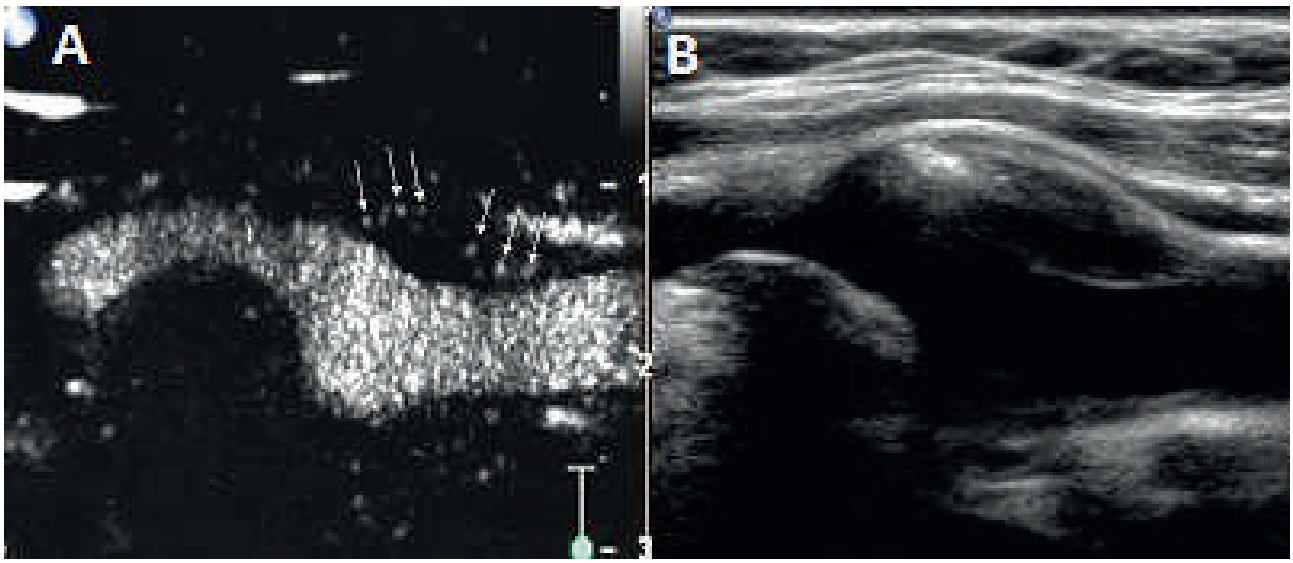
Contrast-enhanced ultrasound has been relatively wide used for several decades in cardiology, and it can provide real time spatial and temporal resolution to define anatomy and tissue perfusion and also allowing vascular tissue imaging [18, 19]. The diameter of ultrasound contrast agents is 1–4 μm corresponding to half of an erythrocyte and it is possible to transit this throughout the capillary system, providing a unique opportunity to quantify microcirculatory blood flow. The contrast agents have to be used in conjunction with ultrasound machines with harmonic imaging frequencies. Tissue perfusion imaging within the microvasculature is detected by identifying the oscillating movements of the microspheres while they move through the capillary vessels: the microspheres behave as resounding targets while receiving and reflecting the harmonic frequencies from/to the ultrasounds [1, 20]. CE-Ultrasounds is emerging as a powerful research tool in atherosclerosis allowing better assessment of carotid lumen and surface, near wall evaluation of intima-media-thickness, and adventitial and plaque neovascularisation [1, 20]. Its use in clinical research protocols has been proposed in a well designed protocol published recently in Stroke where the authors could demonstrate in 147 consecutive patients with atherosclerotic plaques that adventitial vasa vasorum and plaque neovascularisation were directly associated with cardiovascular disease and events (fig. 4) [20].

High-resolution plaque MRI

Magnetic resonance imaging is an emerging method for the assessment of carotid atherosclerosis: by using dedicated surface radiofrequency coils, it may provide high-resolution images of the vessel wall. The image's quality may be optimised by correct preparation of the patient, adequate positioning of the coils, and by the use of pulse sequences with signal suppression of the flowing blood. This allows sub-millimeter voxels

Figure 4

Plaque at the origin of internal carotid artery on B-mode US-imaging (B). Corresponding imaging on contrast-enhanced US (CEUS) (A). (Courtesy PD Dr. Daniel Staub.)



(i.e., volume-pixels) to be obtained [22]. The decrease in signal-to-noise ratio induced by decreasing the voxel size is compensated by the use of phased-array surface coils. Using distinct pulse sequences it is possible to obtain so-called "black blood" images and therefore a good contrast between lumen and the arterial wall. In conjunction with high-resolution time of flight (TOF), bright blood sequences, it is possible to obtain a better definition of the vessel wall with its components. High-resolution MRI is a formidable clinical research tool for measuring plaque progression and/or regression and for studying *in vivo* distinct plaque components [8, 21, 22].

Contrast-enhanced plaque MRI

Dynamic contrast enhanced (DCE) MRI

This technique has been extensively and successfully used for determination and quantification of vascularity of tumours before and after anti-angiogenic treatments. Its application for quantifying plaque neovascularisation has been proposed in animal and human studies [23]. This method uses serial acquisition of MR images with high temporal resolution before and after the injection of Gadolinium (Gd-DPTA): DCE-MRI encompasses the examination of the kinetics of Gadolinium in regions of interest and using appropriate data modelling, which allows the calculation of the extent and permeability of the neovasculature (K trans) [23, 24]. In an ongoing study we are comparing the value of CE-ultrasounds in estimating vessel wall vascularisation with the results obtained by dynamic contrast MRI as a reference method.

Static (late) contrast enhanced MRI

Very recently researchers from Johns Hopkins University have been able to demonstrate the role of detecting adventitial (late) contrast enhancement as a useful marker of vessel wall neovascularisation [25]. The adventitial layer is the major source of vasa vasorum. These authors were able to show how the presence of adventitial (late) enhancement and/or vessel wall haemorrhage was associated with previous cerebrovascular events. When proven in prospective studies these features, together with CE-ultrasounds, might serve as individual markers of plaque instability.

Positron-Emission-Tomography (PET)

^{18}FDG uptake

Recent PET studies in both animal models and humans have shown increased ^{18}FDG uptake by atherosclerotic plaques. It has been suggested that, in the carotid artery, ^{18}FDG may allow detection of active atherosclerotic plaques, thus discriminating between vulnerable and relatively stable lesions [26]. Histological analysis has shown that ^{18}FDG co-localises with macrophage accumulation in the plaque. Initial studies in humans have demonstrated a role for ^{18}FDG PET integrated with computer tomography (CT) imaging in the detection of inflammatory plaques in various vascular territories. Of note, ^{18}FDG uptake provides distinct and complementary information on plaque inflammation compared with MRI. While MRI assesses plaque size and composition (e.g., fat-rich areas, calcifications), ^{18}FDG uptake reflects metabolic processes associated with macrophage accumulation and inflammation.

However, since FDG is accumulated by any metabolically active cell some authors have concerns about the specificity of FDG PET for the detection of inflammatory cells in the atherosclerotic plaque, and these concerns are supported by observations in murine and rabbit atherosclerosis where ^{18}FDG uptake is poorly correlated with macrophage-positive areas in atherosclerotic plaques when compared with histopathological analyses [1, 27].

Moreover, the high background due to myocardial FDG uptake limits the use of FDG PET in the coronary circulation as a consequence of spillover artefacts. These limitations have prompted a search for more specific radiopharmaceuticals for imaging vascular inflammation like the synthetic isoquinoline derivate PK 11195 which binds selectively to peripheral benzodiazepine receptor (PBR) which is highly expressed in activated monocytes [1, 28].

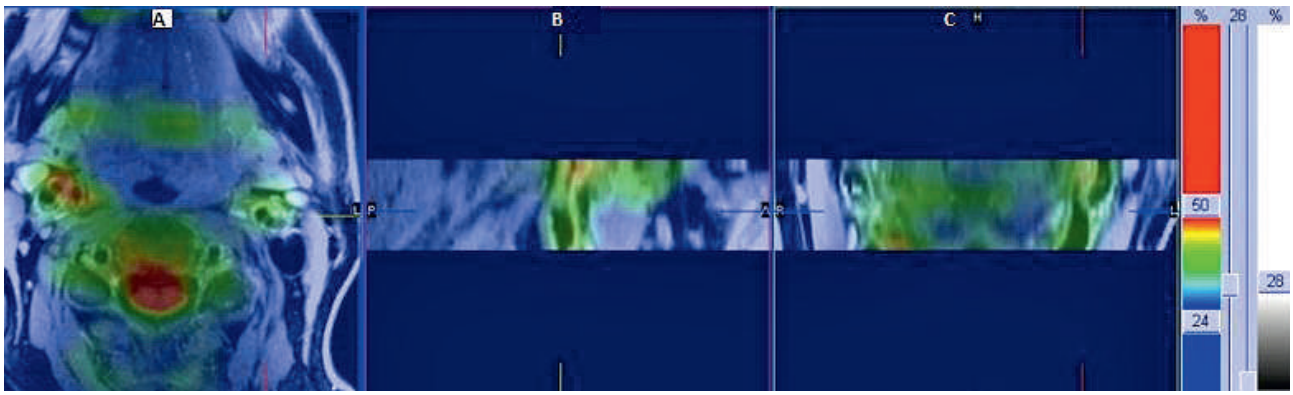
Hybrid imaging

Hybrid imaging may constitute an attractive approach aiming at combining distinct information about the vessel wall including wall morphology and activity [29]. Several years ago, we successfully introduced fusion imaging of hr-MRI plaque imaging with ^{18}PET-CT, therefore combining the method with the best spatial resolution (high-resolution plaque MRI) with the method best characterising the inflammatory activity within the vessel wall (^{18}PET-CT). Figure 5 shows the fusion MRI/PET/CT images of both atherosclerotic carotid arteries in short axis in a patient with a recent right hemispheric ischaemia: a strong enhancement of ^{18}PET-CT is appreciable only at the right symptomatic carotid artery [30]. A joint running project between the CHUV and our cardiovascular unit aims at visualisation of both plaque inflammation and plaque neovascularisation by hybrid imaging including ^{18}PET-CT and Galllium PET, respectively, in patients undergoing carotid endarterectomy, whereby histopathology is used in this study as gold-standard.

Conclusion

This review focusing on our imaging research during the last 15 years underlines the usefulness of studying non-coronary arteries in order to gain a better understanding of the dynamic process of atherosclerosis *in vivo*. Although the majority of the investigative methods remains immature for the time being and reserved to human research purposes, the huge progress in our

Figure 5
The figure shows the hybrid fusioned image of high-resolution MRI and ^{18}FDG PET-CT in a patient with a recent right-hemispheric ischaemic attack. Panel A shows the short axis at the level of the bifurcation of the carotid arteries. In spite of the presence of bilateral atherosclerotic plaques ^{18}FDG-enhancement is present only at the right carotid bifurcation; whereas, no enhancement is present on the contra-lateral carotid bifurcation. The same finding with ^{18}FDG-enhancement is visible on the long-axis view of the right carotid artery (panel B). No enhancement is visible on the long-axis of the left carotid bifurcation. (Courtesy Prof. L. Giovanella, Dr. Luca Ceriani)



understanding of atherosclerosis and the rapid translational of research of technology from bench to bedside, will probably soon open new diagnostic, preventive, and therapeutic avenues which should us allow to better manage patients with atherosclerosis.

References

1 Gallino A, Stuber M, Crea F, Falk E, Corti R, Lekakis J, et al. "In vivo" imaging of atherosclerosis. Atherosclerosis. 2012 May 8 [Epub ahead of print].
2 Dotter CT, Judkins MP. Transluminal treatment of arteriosclerotic obstruction. Circulation. 1964;30:654.
3 Grüntzig AR, Hopff H. Perkutane Rekanalisation chronischer arterieller Verschlüsse mit einem neuen Dilatationskatheter. Dtsch Med Wochenschr. 1974;99:2502–5.
4 Baumgartner I, Pieczek A, Manor O, Blair R, Kearney M, Walsh K, Isner J. Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. Circulation. 1998;97:1114–23.
5 Corti R, Ferrari C, Roberti M, Alerci M, Pedrazzi PL, Gallino A. Spiral computed tomography: a novel diagnostic approach for investigation of the exteacranial cerebral arteries and its complementary role in duplex ultra-sonography. Circulation. 1998;98(10):984–9.
6 Wyttenbach R, Gallino A, Alerci M, Mahler F, Cozzi L, Di Valentino M, et al. Effects of percutaneous transluminal angioplasty and endovascular brachytherapy on vascular remodeling of human femoropopliteal artery by noninvasive magnetic resonance imaging. Circulation. 2004; 110:1156–61.
7 Corti R, Wyttenbach R, Alerci M, Badimon JJ, Fuster V, Gallino A. Images in cardiovascular medicine. Effect of percutaneous transluminal angioplasty on severely stenotic femoral lesions: in vivo demonstration by noninvasive magnetic resonance imaging. Circulation. 2002;106: 1570–1.
8 Bianda N, Di Valentino M, Périat D, Segatto JM, Oberson M, Moccetti M, et al. Progression of human carotid and femoral atherosclerosis: a prospective follow-up study by magnetic resonance vessel wall imaging. Eur Heart J. 2012;33:230–7.
9 Libby P. Inflammation in atherosclerosis. Nature. 2002;420:868–74.
10 Falk E. Unstable angina with fatal outcome: dynamic coronary thrombosis leading to infarction and/or sudden death. Autopsy evidence of recurrent mural thrombosis with peripheral embolization culminating in total vascular occlusion. Circulation. 1985;71:699–708.
11 Davies MJ, Thomas AC. Plaque fissuring – the cause of acute myocardial infarction, sudden ischaemic death, and crescendo angina. Br Heart J. 1985;53:363–73.
12 Sanz J, Fayad ZA. Imaging of atherosclerotic cardiovascular disease. Nature. 2008;451:953–7.
13 Redgrave JN, Lovett JK, Gallagher PJ, Rothwell PM. Histological assessment of 526 symptomatic carotid plaques in relation to the nature and timing of ischemic symptoms: the Oxford plaque study. Circulation. 2006;113:2320–8.
14 Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory Enlargement of Human Atherosclerotic Coronary Arteries. N Engl J Med. 1987;316:1371–5.
15 Montecucco F, Lenglet S, Gayet-Ageron A, Bertolotto M, Pelli G, Palombo D, et al. Systemic and intraplaque mediators of inflammation are increased in patients symptomatic for ischemic stroke. Stroke. 2010; 41:1394–404.
16 Cybulsky MI, Iiyama K, Li H, et al. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. J Clin Invest. 2001;107:1255–62.
17 Moreno PR, Purushothaman KR, Fuster V, Echeverri D, Truszczynska H, Sharma SK, et al. Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. Circulation. 2004;110:2032–8.
18 Gramiak R, Shah PM. Echocardiography of the aortic root. Invest Radiology. 1968;3:356–66.
19 Feinstein SB, Coll B, Staub D, Adam D, Schinkel AF, ten Cate FJ, et al. Contrast enhanced ultrasound imaging. J Nucl Cardiol. 2010;17: 106–15.
20 Staub D, Patel MB, Tibrewala A, Ludden D, Johnson M, Espinosa P, et al. Vasa vasorum and plaque neovascularization on contrast-enhanced carotid ultrasound imaging correlates with cardiovascular disease and past cardiovascular events. Stroke. 2010;41:41–7.
21 Cai J, Hatsukami TS, Ferguson MS, Kerwin WS, Saam T, Chu B, et al. In vivo quantitative measurement of intact fibrous cap and lipid-rich necrotic core size in atherosclerotic carotid plaque: comparison of high-resolution, contrast-enhanced magnetic resonance imaging and histology. Circulation. 2005;112:3437–44.
22 Corti R, Fuster V, Fayad ZA, Worthley SG, Helft G, Smith D, et al. Lipid lowering by simvastatin induces regression of human atherosclerotic lesions: two years' follow-up by high-resolution noninvasive magnetic resonance imaging. Circulation. 2002;106:2884–7.
23 Tofts PS, Brix G, Buckley DL, Evelhoch JL, Henderson E, Knopp MV, et al. Estimating kinetic parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer: standardized quantities and symbols. J Magn Reson Imaging. 1999;10:223–32.
24 Dong L, Kerwin WS, Chen H, Chu B, Underhill HR, Neradilek MB, et al. Carotid artery atherosclerosis: effect of intensive lipid therapy on the vasa vasorum – evaluation by using dynamic contrast-enhanced MR imaging. Radiology. 2011;260:224–31.
25 Qiao Y, Etesami M, Astor BC, Zeiler SR, Trout HH 3rd, Wasserman BA. Carotid plaque neovascularization and hemorrhage detected by MR imaging are associated with recent cerebrovascular ischemic events. AJNR Am J Neuroradiol. 2012;33:755–60.
26 Rudd JH, Warburton EA, Fryer TD, Jones HA, Clark JC, Antoun N, et al. Imaging atherosclerotic plaque inflammation with [18F]-fluorodeoxyglucose positron emission tomography. Circulation. 2002;105: 2708–11.
27 Matter CM, Wyss MT, Meier P, Spath N, von Lukowicz T, Lohmann C, et al. 18F-choline images murine atherosclerotic plaques ex vivo. Arterioscler Thromb Vasc Biol. 2006;26:584–9.
28 Canat X, Carayon P, Bouaboula M, Cahard D, Shire D, Roque C, et al. Distribution profile and properties of peripheral-type benzodiazepine receptors on human hemopoietic cells. Life Sci. 1993;52:107–18.
29 Gaemperli O, Bengel FM, Kaufmann PA. Cardiac hybrid imaging. Eur Heart J. 2011;32(17):2100–8.
30 Corti R, Fuster V. Imaging of atherosclerosis: magnetic resonance imaging. Eur Heart J. 2011;32:1709–19.