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STATE OF THE ART REVIEW
Vascular biology and medicine

Distinct inflammatory pathways shape atherosclerosis in different vascular beds

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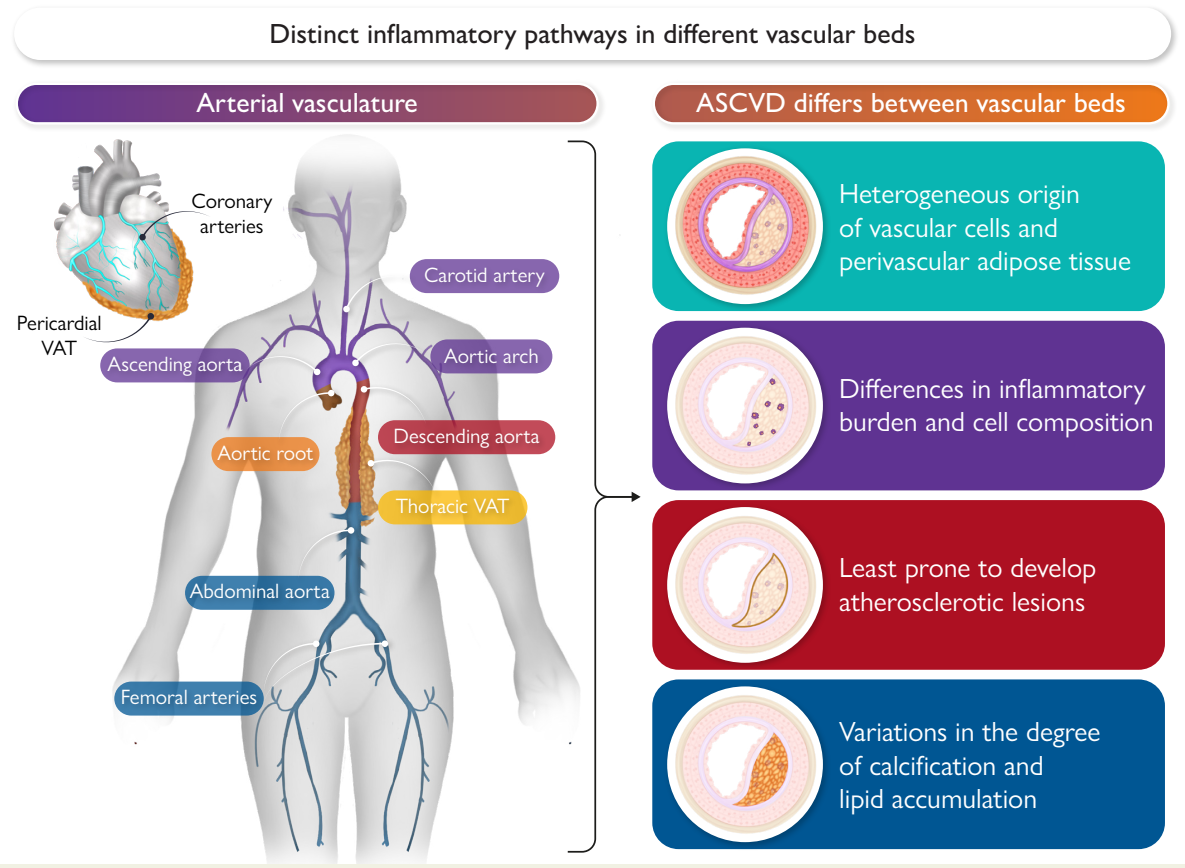
Graphical Abstract



Diverse embryonic origin and heterogeneous atherosclerotic lesions in different vascular beds. VAT, vascular adipose tissue.

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Abstract

Studies suggest varying atherosclerotic cardiovascular disease (ASCVD) prevalence across arterial beds. Factors such as smoking expedite ASCVD progression in the abdominal aorta, while diabetes accelerates plaque development in lower limb arteries, and hypertension plays a significant role in ASCVD development in the coronary and carotid arteries. Moreover, superficial femoral atherosclerosis advances slower compared with atherosclerosis in coronary and carotid arteries. Furthermore, femoral atherosclerosis exhibits higher levels of ossification and calcification, but lower cholesterol concentrations compared with atherosclerotic lesions of other vascular beds. Such disparities exemplify the diverse progression of ASCVD across arterial beds, pointing towards differential mechanistic pathways in each vascular bed. Hence, this review summarizes current literature on immune-inflammatory mechanisms in various arterial beds in ASCVD to advance our understanding of this disease in an aging society with increased need of vascular bed and patient-specific treatment options.

Keywords Atherosclerosis • Cardiovascular disease • Carotid • Coronary • Femoral arteries • Inflammation • Calcification

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Introduction

Arteries are blood vessels that form an essential component of the circulatory system, responsible for transporting oxygen-rich blood from the heart to various tissues and organs throughout the body. These muscular and elastic arteries are designed to withstand and control blood pressure and feed the blood all the way to capillary networks to ensure the exchange of oxygen, nutrients, and waste products at the cellular level. The aorta and carotid arteries are elastic arteries with high concentrations of elastic fibres in the tunica media, allowing them to expand during systole and contract during diastole, while brachial, coronary, radial, and femoral arteries are medium-sized vessels, containing more connective tissue and fewer elastic fibres than aorta and common carotid artery.[1] Altogether they represent the arterial tree which can become prone to develop atherosclerotic cardiovascular disease (ASCVD). Atherosclerotic plaques develop from a well-defined arterial vessel wall to form a benign tumour-like conglomerate of stromal and immune cells. These atherosclerotic lesions form in the inner layer of large arteries at predilection sites such as curvatures and branch points. Advanced atheromas are morphologically characterized by a lipid-rich core with necrotic cells shielded by a fibrous cap and a single layer of endothelial cells (ECs). The development of atherosclerotic lesions is triggered by a number of risk factors including hypertension, smoking, and metabolic disturbance ultimately fuelling endothelial dysfunction and the exudation and retention of plasma lipids in the subendothelial space. As a consequence to endothelial activation, chemokines guide myeloid cells including neutrophils and monocytes to infiltrate the vessel wall.[2] These cells oxidize lipoproteins, engulf them, and ultimately become activated and secrete pro-inflammatory cytokines. Production of cytokines and the ensuing cell death set off a vicious cycle turning developing plaques into complex hyper-inflamed lesions, that—when ruptured—appear clinically as myocardial infarction or stroke.[3] While such pathogenesis is broadly applicable to the various vascular beds, there are indications of distinct immune and inflammatory processes in carotid arteries, aorta, coronary, and femoral arteries. These differences in atherosclerotic lesions between various arterial beds are believed to be the result of a complex interplay of haemodynamic forces, local cellular (e.g. differences in vascular smooth muscle cell [VSMC] origin[4] and phenotype[5] or foam cell content and macrophage phenotype[6]) and molecular environments, structural characteristics of the arterial walls, genetic[7–10] and epigenetic factors, and external influences.[11] However, conclusive comparative studies particularly evaluating distinct vascular bed-specific differences are scarce at this point. This review summarizes the current understanding of differences in atherosclerotic lesion biology across vascular beds paying particular attention to lesional cell composition, potential inflammatory biomarkers and insights from single-cell sequencing studies (*Figure 1*, *Table 1*, *Graphical Abstract*). Notably, we have not addressed in-stent atherosclerosis, transplant atherosclerosis, valve disease, and aneurysms of any kind in this review.

Vascular bed-specific lesion composition—lessons from histological examination

Histological and microscopy analysis of atherosclerotic lesions in different vascular beds has been performed for decades. However, comparative literature of more than two beds, large cohort studies, and comparable tissue preparation and patient characteristics are scarce and limit the possibility of generalizing findings (i) to define true bed-specific lesion characteristics and (ii) to draw therapeutic conclusions to personalize treatment options. Nevertheless, the following section summarizes the most relevant findings of vascular bed-specific ASCVD.

Prevalence of foam cells and lipid-rich necrotic cores is increased in human carotid artery lesions, compared with coronary and particularly in comparison to femoral arteries.[5,26,46] There is also a higher prevalence of intraplaque haemorrhage in human carotid arteries compared with femoral arteries. Carotid artery plaques exhibit thicker fibrous caps, and more calcification compared with coronary and aortic but not femoral artery plaques.[5,13] Still, calcification of carotid artery plaques does not necessarily correlate with increased risk of ischaemic stroke as reported by a recent meta-analysis showing a negative relationship between calcified plaques and ipsilateral ischaemia.[55] The latter may be explained by differential expression of microRNAs[56,57] modulating lesion stability or the type of calcification[58,59] (hydroxyapatite [HA] vs. calcium oxalate [CO]) and not only patterns (micro- vs. macrocalcification) of calcium deposition[60] in general shaping lesion fate and thereby disease risk. Another structure contributing to differences in lesion development across vascular beds in humans is the vasa vasorum network. These blood vessels supplying the walls of larger arteries may be crucial in contributing to the inflammatory process by import of inflammatory mediators fostering plaque development and instability. The vasa vasorum network is largest in coronary arteries and was shown to have significant implications in coronary, carotid, and aortic atherosclerosis compared with femoral ASCVD in humans.[26] In mice, studies on plaque microvessels have been limited due to their low incidence in mouse models of atherosclerosis and most likely also because of inadequate

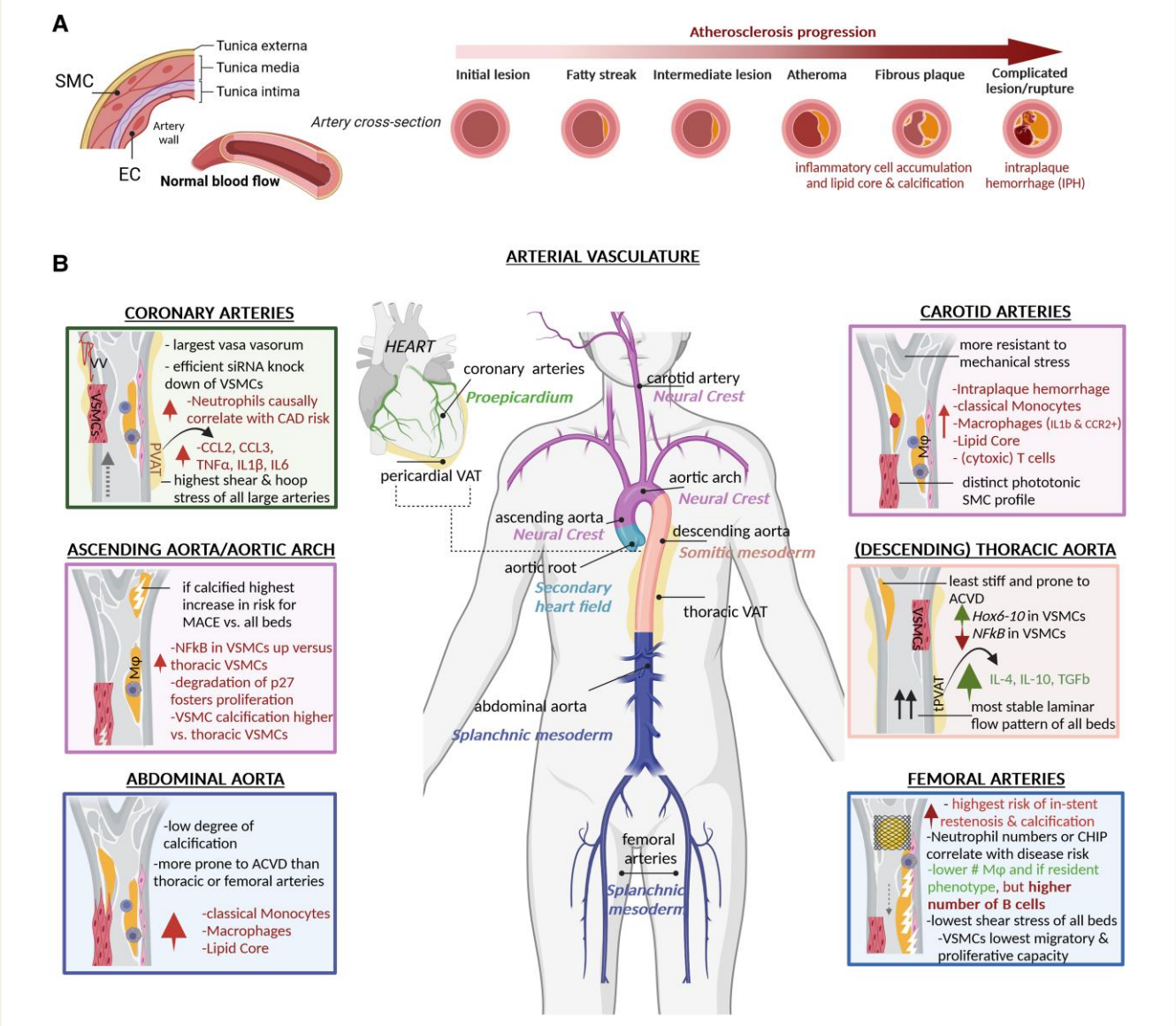

Figure 1 Overview of the most prominent differences in human atherosclerotic lesions in different vascular beds. (*A*) Schematic drawing of an arterial wall and general characteristics of progressing atherosclerotic cardiovascular disease in cross sections of an arterial vessel. (*B*) A human being with arterial tree and heart. Individual arteries are named in black while embryonic origin of vascular smooth muscle cells is added in colour underneath and corresponds to the colour of the respective arterial section of the tree. Boxes left and right summarize individual lesion characteristics of each bed and are framed in the colour of vascular smooth muscle cell origin. In addition, thoracic and pericardial vascular adipose tissues are indicated. ASCVD, atherosclerotic cardiovascular disease; CAD, coronary artery disease; EC, endothelial cell; IL, interleukin; MACE, major adverse cardiovascular events; NF-κB nuclear factor-κB; siRNA, small interfering RNA, SMC, smooth muscle cell; TNF, tumour necrosis factor; tPVAT, thoracic periaortic vascular adipose tissue; VAT, vascular adipose tissue; VSMC, vascular smooth muscle cell; Mϕ, macrophage. Figure was made with Biorender.com

detection tools that lacked the resolution and multi-probe detection ability. Studies applying multiphoton laser scanning microscopy however were able to show angiogenic activity in carotid arteries of old *Apoe*$^{-/-}$ mice and further recapitulated distinct functional characteristics, including blood flow, leukocyte adhesion, and permeability changes aligning with findings in humans.[61] Nevertheless, it still remains debatable how much studies in small rodent models can contribute to our understanding of the role of vasa vasorum in ASCVD in humans.[62] Differences in miRNA expression between vascular beds were also detected in human femoral atheroma which showed higher miR-27a-5p and miR-139-5p expression compared with abdominal aorta and carotid artery.[54] In addition, Kayser *et al.*[22] report that expression of sugar receptors, as assessed by labelled neoglycoproteins or sulphated polysaccharides, may be of particular importance in the development of ASCVD in the coronary and carotid compared with pulmonary or femoral arteries. Femoral artery plaques are most fibrous and show the highest degree of calcification (sheetlike calcification, nodular calcification, and osteoid metaplasia) among all vascular beds.[5,11,26,46–49] Nevertheless, enhanced calcification in all large vascular beds (coronary and carotid arteries and aortic arch) and particularly in the aortic arch are associated with an increased risk of all cause and cardiovascular mortality.[63] Hence, discrepancy in e.g. foam cell formation with carotid arteries depicting the

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Table 1 Summary of structural and cellular alterations of different vascular beds in ASCVD

Vascular bed	Embryonic origin VSMCs	Vascular bed-specific characteristics		
		Structural	Cellular	Other
Carotid arteries	Neural crest[4,12]	• More prone to intraplaque haemorrhage[5,13] • Elastic artery[5] • Most resistant to mechanical trauma[14,15]	• Most prone to foam cell formation[5,11,16,17] • More IL1β positive macrophages[18,19] • More $CD14^{high}CD16^{low}$ classical monocytes[18] • Deregulation of miR-29 and miR-29[20] • Lesions enriched in CCR2+ macrophages[18,21] • Lesions rich in macrophages and cytotoxic T cells, low number of B cells[18,19]	• Sulfated polysaccharides[22] • Distinct photonic profile[23] • Signals from both sympathetic and parasympathetic nerve fibres[24,25]
Coronary arteries	Proepicardium[4,12]	• Largest vasa vasorum[26] • In between muscle and elastic artery[27] • Highest shear and hoop stress among all beds[28,29] • Pericoronary adipose tissue (cPVAT) derived from splachnic mesoderm[30,31]	• VSMCs are more susceptible to gene knock down by siRNA treatment[32] • Adipocytes of cPVAT express high levels of CCL2, CCL3, TNFα, IL1β, and IL6[31,33] • Increased neutrophil numbers causally correlate with disease risk[34] • In non-diseased arteries VSMCs are identified by FN1 and VCAN and fibroblasts by C7 & PTN expression[35]	• Sulphated polysaccharides[22] • Signals from both sympathetic and parasympathetic nerve fibres[24,25]
Aortic root	Secondary heart field[4,12]			
Ascending aorta	Neural crest[4,12]		• In non-diseased arteries VSMCs are identified by CYTL1 and DPT expression[35] • In non-diseased arteries fibroblasts are identified by IER3 and NR4A1[35]	• Aorta receives a dominant sympathetic nerve innervation[24,25]
Aortic arch	Neural crest[4,12]		• High p27 expression in VSMCs[36] • VSMCs calcify earlier than those of the descending aorta[37]	
Descending (thoracic) aorta	Somitic mesoderm[4,12]	• Least prone to develop lesions[38] • Thoracic periaortic PVAT (tPVAT) mixed origin[39,40] • tPVAT secrets high levels of IL10 and IL4[41] • tPVAT exerts atheroprotection[42] • Most stable laminar flow pattern[43,44]	• VSMCs express high levels of Hox6-10, less Nfkb activity[45]	
Abdominal aorta	Splanchnic mesoderm[4,12]	• Elastic artery[5]	• Prone to foam cell formation[5,38] • Less prone to calcification[5]	
Femoral arteries	Splanchnic mesoderm[4,12]	• Highest degree of calcification[5,11,26,46–49] • Muscular artery[5] • Least prone to rupture, but highest risk of in-stent restenosis[14,50] • Variable blood flow[51,52] • Lowest shear stress among all beds[28]	• Least prone to foam cell formation[11,47] • Low p27 expression in VSMCs, TFGbR1 overexpression in VSMCs[32] • More anti-inflammatory foam cell-like macrophages, TREM2-positive and resident LYVE1-positive macrophages[18] • CCR2+ myeloid cells diminished • Increased neutrophil numbers causally correlate with disease risk[34] • Aberrant clonal haematopoiesis confers a greater risk of developing femoral ASCVD[53] • Least number of macrophages and cytotoxic T cells, but high number of B cells[18,19]	• Higher prevalence of miR-27a-5p and miR-139-5p[54] • Deregulation of let 7e, miR-27b, miR-130a, and miR-210[20]

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highest and femoral arteries containing the least amount of foam cells may be due to the fact that the common carotid artery is an elastic artery, similar to the abdominal aorta which is also more prone to foam cell formation.[5] Instead, proximal parts of the coronary arteries and the carotid bifurcation are in between elastic and muscular artery types but also develop foam cell rich lesions and lipid cores early on.[27] Noteworthy, the internal carotid artery and the superficial femoral arteries are both muscular arteries, but foam cell lesions are more common in the internal carotid artery.[27] These findings suggest that not only the type of artery (elastic vs. muscular) determines the extent of foam cell formation, calcification and lesion composition;—instead VSMC origin[4,5,37,64] may play a dominant role in determining atheroprone arterial regions from others (*please refer to paragraph below for VSMC heterogeneity in vascular beds*). This notion is supported by a very early study in dogs using aortic homograft transplantation to examine the responses of different aortic segments to a high fat atherogenic diet.[38] Segments of atherosclerosis-resistant thoracic aorta were transplanted into atherosclerosis-susceptible abdominal aorta and vice versa. After one year, the atherosclerosis-resistant thoracic aorta segments in the abdominal aorta remained lesion-free, while the adjacent? abdominal aorta developed severe atherosclerosis. Conversely, susceptible segments in the thoracic aorta still developed severe atherosclerosis, despite the lesion-free thoracic aorta around them.[38] The latter rather argues against extrinsic factors determining the degree of susceptibility of arteries to ASCVD. Thus, far it remains one of the few studies investigating this hypothesis in a larger animal model in a long-term study set-up. Discrepancy also exists on the location of early lesion appearance. While autopsy analyses describe onset of lesion development to be later in femoral arteries[47] than in common carotid or coronary vessels, non-invasive ultrasound analysis in a large cohort of middle-aged men suggest sub-clinical atherosclerosis to be more prevalent in femoral arteries,[65] but femoral lesions are described to be less prone to rupture.[47] These contrary findings may be due to heterogeneous ways of sample collection, patient inclusion and diagnostic approaches applied. Undoubtful, the risk of in-stent restenosis is highest in femoral arteries[14,50] compared with coronary[66] or carotid[67] arteries. The latter might be due to a larger diameter of femoral than coronary and carotid arteries which could lead to different haemodynamic stresses and healing responses.[66] Femoral arteries also exhibit a more variable blood flow due to leg movements and a higher likelihood of disturbed flow, increasing the risk of endothelial damage and restenosis.[51] Additionally, the femoral artery experiences lower and variable shear stress and abnormal patterns of multidirectional wall shear stress were shown to be associated with lumen remodelling within 1-year post-intervention and to promote VSMC proliferation and neointimal hyperplasia, key factors in restenosis.[52] Femoral and carotid vessels respond differently to endarterectomy procedures as shown by Cunnane *et al.*[14] Here, the authors compare mechanical properties and composition of carotid and femoral plaques. Vessel rupture upon stretching, strength, and stiffness are significantly higher in carotid arteries suggesting that carotid arteries are more resistant to mechanical trauma,[14] which would support earlier studies showing that a reduced ability to undergo circumferential extension prior to tissue failure correlates with the degree of restenosis observed following endovascular intervention.[15] Different mechanical properties of various arteries were also reported by Dinardo *et al.*[68] demonstrating that femoral, renal, abdominal aorta, carotid, mammary, and thoracic aorta exhibited a significantly descending order of stiffness. Their VSMC mechanical data correlated with the vessel percentage of elastin and amount of surrounding extracellular matrix, which decreased with the distance from the heart.[68] Proteomics analysis of VSMCs carried out in parallel in the same study revealed a significantly higher amount of cytoskeleton proteins, including actin, microtubule-associated proteins but also members of focal adhesions like vinculin and alpha-actinin in VSMCs from the thoracic aorta. Femoral artery VSMCs expressed significantly more proteins involved in cell cycle network.[68] However, thoracic and femoral VSMCs contained similar numbers of cells in each stage of cell cycle and femoral VSMCs, despite having more protein content associated with cell cycle, did not proliferate more.[68] At this point, it is not clear how these differences in basic protein content relate to ASCVD-relevant functional differences between the two VSMC subsets.

Despite these distinct differences, a large meta-analysis of over 22,000 patients suggests that evaluation of carotid artery disease does also reflect on coronary artery disease (CAD): The authors show that carotid intima-media thickness (IMT) was increased in a linear manner proportional to the severity of CAD and carotid IMT also correlated with the number of diseased coronary vessels; and carotid IMT $\geq$ 1.0 mm rather than plaque presence in carotid arteries per se was the best predictor of CAD.[69] The authors further report a moderate correlation between the degree of carotid and coronary stenosis as well as calcification.[69] Similar results are reported by Cohen *et al.*[70] performing carotid artery ultrasound and coronary artery computed tomography angiography in 150 out-clinic patients revealing a correlation between carotid plaque and increased carotid IMT and the presence and severity of coronary calcification.[70] In line with these results, another study investigating 181 patients with unstable and 92 with stable angina pectoris scheduled for coronary bypass surgery found that complicated and unstable atherosclerotic plaques in carotid arteries are much more common in patients with unstable angina pectoris along with significantly enhanced levels of C-reactive protein indicating that plaque instability in coronary arteries may also involve the carotid arteries in a pan-vascular inflammatory plaque activation scenario.[71] Similarly, patients with femoral artery disease have an elevated risk of sub-clinical CAD and vice versa.[72]

Taken together, these observational studies confirm a pronounced heterogeneity between different vascular beds and emphasize distinct characteristics, including enhanced macrophage accumulation in carotid and pronounced calcification in femoral arteries (*Figure 1*, *Table 1*). Nevertheless, mechanistic insights on which mechanisms these differences are based on remain thus far largely elusive.

Haemodynamic differences and endothelial phenotypes across vascular beds

The arterial endothelium forms the inner layer of the artery and is hence in constant interaction with the blood flow and its haemodynamic forces.[28] The main haemodynamic force impacting on ECs is shear stress, the frictional force generated by the viscosity of blood and its tangential flow over the endothelial surface.[73] Shear stress is an important regulator of key endothelial functions including cell fate decisions, permeability, and matrix production. Stable, laminar flow is in principle atheroprotective by enhancing tight junction stability and adherens junction integrity through enhanced expression of junctional molecules and enforcing their signalling pathways.[43,44] Unstable, disturbed flow on the other hand links with atherogenic endothelial responses including senescence, apoptosis, upregulation of adhesion molecules and increases in endothelial permeability.[74] Given the importance of shear

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stress in regulating endothelial function it is important to note that haemodynamic patterns greatly vary across the various arterial beds affected by atherosclerosis. In straight regions of large arteries, a stable, laminar flow dominates the flow pattern with a shear stress of 5–20 dyn/cm^2. In the context of vascular bed-specific lesion formation, it is important to note that the shear stress is lowest in the superficial and common femoral artery (4–7 dyn/cm^2), followed by the carotid artery (10–15 dyn/cm^2). Shear stress is highest in the coronary arteries ranging from 6 to 45 dyn/cm^2.[28] Of note, shear stress is not the only biomechanical factor impacting different vascular beds. Another example is circumferential ('hoop') stress, which is a result of the vessel's resistance to bursting effect of the applied internal pressure. Its magnitude greatly depends on the vessel diameter and is thus much higher in coronary arteries as compared with the aorta.[29] Such differences as well as the fact that femoral and carotid arteries are perfused during the systole, the coronaries during the diastole, may induce differential responses in the three endothelial beds. Indeed, recent advances in single-cell RNA-seq have revealed differences in endothelial phenotypes across various vascular beds[75] and shed light on the shear stress-induced endothelial responses.[76] Yet, as of now, there is no detailed analysis of ECs in carotid, femoral, and coronary arteries available that would reveal different phenotypes and reactions in responses to vascular-bed-specific shear patterns.

VSMC heterogeneity across vascular beds

Lineage tracing studies have demonstrated that VSMCs originate from multiple different sources during development,[4,77] suggesting that variations in the embryological origins of VSMCs may contribute to arterial bed-specific calcification[37] and local susceptibility to atherosclerosis.[14,38,47,48] For instance, VSMCs in the ascending aorta, aortic arch, and pulmonary trunk are derived from neural crest cells while descending aortic VSMCs originate from the somitic mesoderm and VSMCs in the abdominal aorta and femoral arteries derive from the splanchnic mesoderm while progenitors of coronary VSMCs are located in the proepicardium, which originates from the lateral plate mesoderm (*Figure 1*, *Table 1*).[4,12] Studies examining differences in VSMC function in ASCVD with respect to their embryonic origin are scarce. The few studies comparing VSMC function of different vascular beds with respect to ASCVD often retrospectively attribute these differences to the diverse embryonic origin. Trigueros-Motos *et al.*[45] for example report that homeobox paralogous genes 6 to 10 (Hox6–10) are more abundantly expressed in VSMCs from the thoracic aorta compared with VSMCs from the atherosclerosis-susceptible aortic arch.[45] The latter could be shown in VSMCs from mice, rats, and pigs and also in human embryonic stem cells differentiated into neuroectoderm–VSMCs and somitic mesoderm–VSMCs, which give rise to aortic arch and thoracic aorta VSMCs, respectively. Further, thoracic VSMCs have lower activity of the pro-inflammatory and proatherogenic nuclear factor-κB (NF-κB) and lower expression of NF-κB target genes. Based on these results the authors propose that embryonically imprinted differential Hox expression may contribute to the establishment of distinct regional molecular signatures in the adult vasculature that modulate pathophysiological processes in ASCVD.[45] Apart from differences in gene expression single-cell photonics (label free) as a discriminator of cell phenotype also revealed differences in medial aortic vs. carotid VSMCs.[23] Sadly, the study does not specify the region of the aorta VSMCs originated from. From here, the authors conclude that these differences in the photonic profile of medial VSMCs between vascular beds indicates the existence of specific subsets of medial VSMCs with particular disease relevant photonic profiles and propose that these differences are due to the divergent embryonic origin of carotid and thoracal aorta VSMCs.[23] Others describe an important role for the mitogen-activated protein kinase pathway in the control of growth suppressor p27Kip1 (p27) expression in aortic arch VSMCs and femoral VSMCs thereby controlling their proliferative and migratory behaviour.[36] The authors report that stable extracellular signal-regulated kinase (ERK)1/2 activation in mitogen-stimulated aortic arch SMC cultures facilitates p27 degradation, thereby favouring their proliferation and migration. In contrast, weaker ERK1/2 activation in femoral VSMCs facilitates higher expression of p27 in these cells thereby hindering their proliferative and migratory responses. The authors argue that these differences in p27 expression are attributable to a different embryonic origin of aortic arch and femoral VSMCs (neural crest and mesoderm, respectively) and may contribute to differences in atherosclerotic lesion composition and development.[36] Similarly, coronary VSMCs do also exhibit a higher migratory and proliferative capacity compared with femoral VSMCs.[78] Down this road Leroux-Berger *et al.*[37] reveal that medial aortic arch VSMCs made up of VSMCs of neural crest origin calcified significantly earlier than VSMCs of the descending aorta, which are somitic mesoderm derived.[37] In line, abdominal lesions are also reported to be less prone to calcification[5] which is however in contrast to femoral arteries which are—like the abdominal aorta—also splanchnic mesoderm derived but described to have the highest degree of calcification.[5] This discrepancy argues against a dominant role embryonic origin in shaping—at least—VSMC calcification patterns in ASCVD. Contrary, another study reports a marked difference in many developmental genes and basal transforming growth factor-beta (TGFβ) signalling or TGFβ sensitivity in femoral arteries compared with carotid and aortic (thoracic and abdominal) smooth muscle cells (SMCs) and reveals overexpression of TGFβR1 in femoral arteries favouring VSMC switching, mineralization, and hence calcification.[5] The authors—at least partly—also attribute these changes to a different embryonic origin of these vascular beds. Interestingly, aortic VSMCs (embryonic origin not clear) are less susceptible to gene knock down by siRNA treatment compared with coronary VSMCs.[32] Nevertheless, if this truly relates to their different embryonic origin remains at this point elusive. To the best of our knowledge, there is no study so far systemically comparing to which extent these differences in embryonic origin dictate human VSMC behaviour in ASCVD across different vascular beds.[77]

Macrophage phenotypes and activation

Classical histological and immunohistochemical analyses have identified macrophages to be the most prominent immune cell in atherosclerotic lesions. Macrophages derive from circulating monocytes that are recruited to plaques in a multi-step cascade involving adhesion molecules such as VCAM1 and chemokines such as CCL2 and CCL5.[79,80] Yet, it is important to point out that entry routes and mechanisms of myeloid cell recruitment identified in mouse models of atherosclerosis may not readily link to human disease as the density of vasa vasorum and the degree of plaque neoangiogenesis is very limited in mouse models.[61,62] Once in the plaque, monocytes differentiate towards macrophages, a population efficiently clearing oxidized lipids from the plaque. In response to lipid uptake and stimuli perceived from the

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microenvironment, macrophages can adopt different phenotypes and functions ranging from pro- to anti-inflammatory.[81] Importantly, the inflammatory status of atherosclerotic lesions differs along the arterial tree (*Figure 1*, *Table 1*). A comparison of femoral and carotid arteries in terms of leukocytic infiltrate revealed a higher macrophage burden in carotid lesions.[16] The higher inflammatory burden in the carotid is corroborated by non-invasive PET-CT analyses of femoral and carotid lesions. Specifically, the uptake of ^{18}fluorodeoxy-glucose was found to be higher in carotid plaques as compared with femoral lesions indicating a higher metabolic and inflammatory activity.[17] To unveil the inflammatory continuum of macrophages across plaques in different vascular beds, a recent study has made use of scRNAseq of CD45-positive leukocytes obtained from femoral and carotid plaques.[18] In agreement with previous histological and imaging studies, femoral plaques contained more anti-inflammatory foam cell-like macrophages, TREM2-positive macrophages and resident LYVE1-positive macrophages. In contrast, IL1β positive macrophages were strikingly diminished in femoral lesions compared with carotid plaques.[18,19] In addition, CCR2^{+} myeloid cells are vastly diminished in plaques obtained from the femoral as compared to the carotid plaque. Given the importance of the CCL2-CCR2 axis in the circadian recruitment of myeloid cells to atherosclerotic lesions,[80] one may speculate that time-of-day optimized neutralization of this axis preferentially improves inflammation in the carotid artery. Of note, these scRNAseq data are in line with flow cytometric analyses of femoral and carotid lesions. There, CD14^{high}CD16^{-} classical monocytes, the progenitors of inflammatory macrophages, are enriched in carotid plaque specimens.[18] While scRNAseq data are important to reveal subtle differences in cell composition and activity, these analyses are devoid of spatial information. Yet, in the context of biology and therapeutic targeting, spatial distribution of macrophage subsets is key and future studies will need to compare the location of different macrophage subsets with plaques obtained from the carotid, the femoral, and the coronary arteries.

Adventitia and perivascular adipose tissue

In arteries, not only the intimal and medial layers exhibit phenotypic differences and respond differentially to challenges across vascular beds, but also the most outer layer, namely the perivascular adipose tissue, displays regional differences.[30] The pericoronary adipose tissue (cPVAT) is derived from the splanchnic mesoderm and is characterized by a gene expression signature with high expression of UCP1, PRDM16, PPARɣ, and the beige adipocyte marker CD137.[31,82] Adipocytes from human cPVAT show remarkable inflammatory responses outcompeting the chemokine expression of subcutaneous or visceral adipocytes obtained from the same individual. Among these chemokines are monocyte-attracting CCL2 and CCL3 as well as pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6.[31,33] The secretion of such inflammation-stimulating factors is one mechanism by which the cPVAT is thought to promote coronary atherosclerosis.[83] Along the aortic tree, the perivascular adipose tissue shows a remarkable heterogeneity, which is also reflected by an ongoing discussion on its embryonic origin.[39,40] The thoracic periaortic PVAT (tPVAT) is characterized by brown adipocytes and the secretion of various anti-inflammatory mediators in mice including IL-10 and IL-4. tPVAT is largely resistant to diet-induced macrophage infiltration and may hence exert protective effects during inflammatory stress.[41] The anti-atherosclerotic effects of tPVAT have been shown in transplantation experiments. Herein, transfer of tPVAT over the infrarenal aorta reduced local plaque burden by 20%, an effect reverted by neutralization of TGFβ. Thus, these data suggest that the atheroprotective effect of tPVAT is centred on a TGFβ driven anti-inflammatory cascade.[42] The perivascular tissue surrounding arteries such as the mesentery, carotid, and femoral arteries is largely reminiscent of white adipocytes and expression of Hoxc8, Tcf21, and dermatopontin.[84] Adipocytes within this PVAT are able to produce large amounts of cytokines including TNF-α and IL-6 as well as chemokines such as CCL2, CCL5, and CX3CL1 and hence are thought to promote local plaque formation.[85,86] Of note, the aforementioned soluble factors have been shown to promote vascular calcification and may hence represent a link between local PVAT activity, and the overt calcification observed in the femoral plaque.[87] As an example, antibody neutralization of TNF-α reduced lesional Wnt signalling and calcification in *Ldlr*$^{-/-}$ mice.[88] Similarly, IL-6 was shown to induce differentiation of VSMCs into osteoblast-like cells and knockdown of CCL2 or its receptor CCR2 prevents calcification in a mouse model of atherosclerosis.[89,90]

The adventitia is also the location of local nerve fibres, with regional variations observed in sympathetic and parasympathetic nerve innervation. Larger arteries such as the aorta receive a dominant sympathetic innervation while mid-sizes arteries such as the coronary arteries and the cerebral receive signals from both sympathetic and parasympathetic nerve fibres.[24,25] Recent work points towards the development of neuroimmune cardiovascular interfaces at sites of atherosclerotic lesions.[91] Of note, axon growth was increased with ageing and was more pronounced at sites of adventitial immune cell aggregates. These neurovascular interfaces also contain sensory neurofibres and the density of these structures increased with ageing. Yet, detailed analysis of regional differences is thus far elusive.

Moreover, the adventitia does not only harbour nerve endings but also connects to the lymphatic system, which includes highly permeable initial lymphatics and larger collecting vessels, crucial for immune surveillance, lipid absorption, and fluid balance. Lymphatic vessels develop from lymphatic endothelial cells (LECs) sprouting from embryonic veins and lymph sacs, with LEC progenitors traced to venous origins, haemogenic ECs, dermal capillaries, non-venous sources, and the second heart field. Most Prox1-positive LECs in embryos originate from the paraxial mesoderm.[92,93] Hence and similar to differences in the embryonic origin of VSMCs, LEC origin may differentially shape vascular bed-specific fluid exchange and thereby impact on ASCVD phenotype in a particular bed. In general, studies in transgenic mice show that impaired lymphatic function accelerates atherosclerosis, while enhanced lymphatic activity protects against it by supporting reverse cholesterol transport and reducing plaque inflammation.[94,95] Therapeutic strategies, like VEGF-C administration, stimulate lymphangiogenesis, and improve lymphatic transport, reducing plaque and inflammation in models of atherosclerosis and post-myocardial infarction.[96,97] Most specific studies currently do exist for cardiac lymphatics showing that cardiac lymphangiogenesis supports recovery after myocardial infarction by alleviating oedema, inflammation, and fibrosis, positioning lymphatic-targeted therapies as promising treatments for ischaemic heart disease.[98,99] Yet, as detailed above stimulating lymphangiogenesis has also proven beneficial in ASCVD without further specification on alterations in different vascular beds. In summary, the impact of angiogenic drugs and in addition modulation of bed-specific lymphatics need to be explored in much more detail to allow for specific targeting.

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Biomarkers

Current guidelines on cardiovascular disease prevention recommend risk assessment using algorithms such as the Systematic Coronary Risk Evaluation or the Framingham Risk Score (FRS).[100] These integrate classical risk factors to stratify patients. However, inflammatory, blood-based biomarkers are not routinely integrated in such score algorithms, although they can deliver an additional degree of sensitivity and specificity. Yet, at this point, these scores nor other soluble blood-based markers can predict site-specific atherosclerotic complications.

In addition, miRNAs have been discussed as soluble biomarkers of ASCVD and vascular bed-specific data suggest common deregulation of miR-21, miR-30, miR-126, and miR-221-3p in all beds while deregulation of miR-21 and miR-29 seems to be specific for carotid atherosclerosis, and let 7e, miR-27b, miR-130a, and miR-210 in femoral artery atherosclerotic disease.[20]

Already in the 1970s associations between leukocyte counts and risk of myocardial infarction have been reported.[101] In recent years, large cohort studies have identified a clear association of high neutrophils counts in the circulation with heightened risk of myocardial infarction and peripheral artery disease.[102] While neutrophil counts robustly predict future cardiovascular events, Mendelian randomization analyses revealed a causal contribution of neutrophils to peripheral artery disease and CAD, hence confirming mechanistic observations made in mice.[34,103] Yet, neutrophils are not a homogeneous population. These cells appear in different flavours reflecting activation status, intrinsic ageing programs, or maturation stages.[104] Future analyses will need to reveal if neutrophil phenotypes allow a better risk prediction than the global neutrophil population and if such can predict site-specific vascular inflammation. Moreover, very recent work has suggested that aberrant clonal haematopoiesis in middle-aged healthy individuals confers a greater risk of developing particularly de novo femoral atherosclerosis in a 6-year period of follow-up. These findings indicate that clonal haematopoiesis unidirectionally promotes atherosclerosis and suggest monitoring of clonal haematopoiesis to be added into strategies for the prevention and early treatment of ASCVD in individuals exhibiting changes in clonal haematopoiesis.[53] As the literature of inflammatory biomarkers for cardiovascular disease is vast, we would like to refer the reader to recent reviews for complete listings of relevant biomarkers.[105,106]

Insights from single-cell sequencing studies

Single-cell technologies have become widely available and enable us to study the transcriptome of tissues at a single-cell resolution. In the CVD arena, single-cell technologies including scRNAseq, snRNAseq, snATACseq, CITE-seq, and spatial transcriptomic approaches have significantly contributed to our understanding of atherosclerosis pathology and have redrawn our understanding of human atherosclerotic disease development.[107]

The increasing availability of scRNAseq data in human atherosclerosis has resulted in a detailed understanding of leukocyte, EC, and VSMC subsets that are present in the plaque, and studies addressing their functions and interactions are underway. We currently identify ~4 macrophage populations, ~8 T cell populations, ~6 EC populations, and up to 6 VSMC subsets that are present and interact in atherosclerotic plaques.[107,108] The majority of these insights are based on data from carotid atherosclerotic plaques, whose tissue is easily accessible through carotid endarterectomies.[21,109–111] Fewer studies focus on coronary atherosclerotic plaques and roughly find similar cell subsets as in carotid atherosclerotic plaques, but comparison is difficult, as coronary plaques still contain the arterial media, whereas carotid endarterectomy plaques do not.[112–114] As atherogenesis and plaque composition are arterial-site specific, more efforts should be undertaken to obtain single-cell data from atherosclerotic plaques from other sites and provide a detailed comparison of differences in cellular subsets and cell-type-specific transcriptomes at these different arterial sites.

Only a few studies address cellular composition of (plaques at) different sites of the arterial tree at a single-cell transcriptional level. Hu *et al.*[35] performed comparative single-cell analyses on the aorta, pulmonary artery and coronary artery. Non-diseased segments of these three arteries were obtained from explanted hearts. The aorta and pulmonary arteries were cut in 1 cm pieces above the valve, and 2.5 cm pieces of the left coronary, right coronary, and circumflex artery were dissected from the epicardium.[35] VSMCs were the major cell population in these non-diseased arteries. Two different synthetic VSMC populations were identified with the VSMC1 (expressing FN1, VCAN) population being dominant in the coronary artery, and the VSMC3 (expressing CYTL1, DPT) in the aorta and pulmonary artery. The VSMC2 cluster, containing contractile VSMCs was similar in all arterial beds, whereas the VSMC4 cluster, which was highly proliferative, was mainly observed in the aorta. Fibroblast populations also differed largely between the different sites. Aorta and pulmonary artery fibroblasts mainly expressed early response genes such as IER3 and NR4A1, whereas coronary arteries contained more inflammatory fibroblasts, expressing C7 and PTN. The four major EC populations could be detected in aorta, pulmonary, and coronary arteries, with a greater presence of the EC3 population (SULF1, END1), a population associated with pro-migratory ECs with endothelial to mesenchymal transition characteristics, in the coronary artery. Immune cells also had a significant presence in these healthy arteries. Four macrophage subsets, including two inflammatory subsets, one resident macrophage subset, and one proliferative macrophage subset were detected, and all subsets were found at similar ratios in all arterial beds. Similar results were obtained from the T cell and NK populations. Four T cell subpopulations and two NK cell populations could be detected but were found in similar ratios across the different arterial beds. These data show that the non-diseased aorta, pulmonary, and coronary artery predominantly differ in their VSMC, fibroblast (predominantly of medial origin), and EC components, and that the immune cell component is similar in a non-diseased stage. Although the major differences in VSMC and fibroblast phenotypes are most likely due to the structure and mechanical properties of the individual arteries, these different subsets may drive atherogenesis differently. The coronary artery is more prone to developing atherosclerosis, which may be reflected by a more pro-inflammatory fibroblast population, as well as the presence of an EC population prone to endothelial-mesenchymal transition.

In another study, Slysz *et al.*[18] were able to compare the transcriptional profile of $CD45^+$ plaque leukocytes between carotid and femoral atherosclerotic plaques.[18] The study was well designed: plaques at both sites were obtained via an endarterectomy procedure, meaning that both plaques were not contaminated by the arterial media, and patients were sex and age matched. All plaques were processed and analysed in a similar way, making this a true comparison between immune cells of carotid and femoral atherosclerotic plaques. In this study, eight macrophage-clusters were determined, and carotid artery plaques were especially enriched in pro-inflammatory foamy $IL1B^+$ macrophages, whereas femoral plaques contained more anti-inflammatory

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foamy macrophages, as well as more LYVE$^+$ tissue resident macrophages. Carotid plaque macrophages have a more pro-inflammatory profile and up-regulate pathways enriched in chemokine and cytokine responses. This was confirmed in a follow-up study, where flow cytometry showed that carotid plaques are highly enriched in CCR2$^+$ macrophages, a macrophage subtype known to drive inflammation and atherosclerosis.[21] The lymphoid population also showed a divergent distribution between carotid and femoral atherosclerotic plaques. Carotid atherosclerotic plaques contained more cytotoxic CD8$^+$ T cells than femoral plaques, and almost all other T cell subsets, expressed genes with a more inflammatory profile in carotid vs. femoral atherosclerotic plaques. Femoral atherosclerotic plaques were highly enriched in B cells, which were balanced in B1 and B2 cells, whereas carotid plaques only had a minor B1 cell fraction. B1 cells are an innate type B cell, known by its production of anti-OSE IgM antibodies, which are protective in atherosclerosis. Similar findings were also reported by Wang *et al.*[19] who performed single-cell sequencing of four femoral plaques and compared these data to single-cell sequencing results of three carotid samples from GSE159677. The authors particularly emphasize that by comparing the proportion of cells in the carotid and femoral artery, it was found that the proportion of T cells, particularly memory and cytotoxic T cells, and the proportion of inflammatory macrophages was much higher in the carotid than in the femoral artery.[19]

These results clearly show that the femoral atherosclerotic plaque has a more homeostatic inflammatory phenotype, whereas carotid atherosclerotic plaques are clearly pro-inflammatory, which fits with the clinical observations that atherosclerotic plaques in the femoral artery are less prone to rupture and more likely to progress gradually than carotid artery plaques.[115–117]

Although these studies confirm the differences in cellular composition and single-cell transcriptomes in atherosclerotic plaques at different sites of the arterial tree, additional in-depth single-cell analyses comparing single-cell data of plaques at different arterial sites are needed to fully comprehend the different mechanisms that drive the divergent pathogenesis at different sites of the arterial tree. The application of spatial transcriptomics, as has been performed in carotid endarterectomy plaques by Sun *et al.*,[118] will prove invaluable for this purpose, as this technique enables single-cell analysis of precise areas of atherosclerotic plaques obtained from different sides of the arterial tree by autopsy or surgery from a single tissue section.

Outlook and conclusion

Current knowledge on vascular bed-specific ASCVD mechanisms is limited, mainly due to a lack of large, standardized cohort studies. Key challenges include patient heterogeneity, limited translation from animal models, varied imaging requirements, funding focus on more common ASCVD types, and logistical and ethical barriers to collaboration and data integration. Overcoming these challenges could provide valuable insights for tailored treatments. Apart from these more structural reasons it is also not clear why some patients present with single-vessel coronary disease, whereas others present with multivessel disease, with varied distribution and plaque burden severity. Similar findings have been described in the carotid circulation, with the majority of patients presenting with unilateral disease vs. the minority presenting with bilateral disease.[119] According to embryonic origin and other aspects of local variation, one could expect that at least for the same artery type susceptibility is comparable. Currently, it remains unclear why this is not the case.

As of now, well-designed single-cell and spatial transcriptomic studies hold a great promise to shed light on mechanistic details underlying the enormous bed-specific heterogeneity of lesion development and progression. Furthermore, it is tempting to speculate that in-depth omics of the thoracal aorta could introduce targets which—when appropriately activated—could generate an atherosclerosis-resistant phenotype. In addition, and not covered in this review due to a lack of original data, such analyses would also need to address sex-specific effects which introduce another layer of complexity in ASCVD in different vascular beds.

Supplementary data

Supplementary data are not available at *European Heart Journal* online.

Declarations

Disclosure of Interest

All authors declare no disclosure of interest for this contribution.

Data Availability

No data were generated or analysed for this manuscript.

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