

The year in basic vascular biology research: from mechanoreceptors and neutrophil extracellular traps to smartphone data and omics

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Abstract

2020 has been an extraordinary year. The emergence of COVID-19 has driven urgent research in pulmonary and cardiovascular science and other fields. It has also shaped the way that we work with many experimental laboratories shutting down for several months, while bioinformatics approaches and other large data projects have gained prominence. Despite these setbacks, vascular biology research is stronger than ever. On behalf of the European Society of Cardiology Council for Basic Cardiovascular Science (ESC CBCS), here we review some of the vascular biology research highlights for 2020. This review is not exhaustive and there are many outstanding vascular biology publications that we were unable to cite due to page limits. Notwithstanding this, we have provided a snapshot of vascular biology research excellence in 2020 and identify topics that are in the ascendancy and likely to gain prominence in coming years.

Keywords

Vascular biology • Mechanoreceptors • NETs • Smartphone data • Omics

1. COVID-19

This year will be remembered for the coronavirus disease 2019 (COVID-19) pandemic, which has had a major impact on all parts of society including scientific research. In addition to its effects on the respiratory system, the virus that causes COVID-19 called severe acute respiratory syndrome coronavirus type 2, (SARS-CoV-2) causes major cardiovascular complications including damage to the heart (e.g. myocarditis, arrhythmias, and myocardial damage) and the vasculature. Endothelial cells (EC) have been implicated in COVID-19 due to their expression of angiotensin converting enzyme 2

(ACE2) which is the receptor for SARS-CoV-2,¹ the prevalence of vasculitis and systemic inflammation (cytokine storm)^{2,3} and disseminated intravascular coagulation in COVID-19^{4,5} and direct evidence of EC infection with SARS-CoV-2.^{6–9} Plasma from critically ill COVID-19 patients was shown to be cytotoxic to human cultivated pulmonary microvascular lung ECs.¹⁰ Besides increased levels of inflammatory cytokines such as interleukin 6 (IL-6) or macrophage chemotactic protein-1 (MCP-1), also high levels of the antifibrinolytic, prothrombotic plasminogen activator inhibitor type 1 (PAI-1), all most likely released from the activated endothelium, were measured in the plasma of patients suffering from COVID-19.¹¹ In rhesus monkeys

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infected with SARS-CoV-2, thrombosis and endothelial damage was seen similar to that observed in humans suffering COVID-19. Underlying mechanisms seem to include activation of the complement systems, platelet activation via the interferon- α pathway, and enrichment in inflammatory cytokines and proinflammatory M1 macrophages.¹² A still elusive aspect of the pathogenesis of COVID-19 infections in humans is the considerably higher death rate in males than in females. A recent review summarizes the role of sexual dimorphism in the regulation of adaptive and innate immunity and discusses current evidence for a possible influence of biological sex on the regulation and the expression of ACE2.¹³ We have recently reviewed the critical role of the vasculature in COVID-19 on behalf of the *European Society of Cardiology (ESC) Council for Basic Cardiovascular Science (CBCS)* and refer the reader to this¹⁴ and related publications.¹⁵ There have been several advances since our review including the observation that SARS-CoV-2 infection involves the high density lipoprotein (HDL)-interacting scavenger receptor class B type 1 (SR-B1).¹⁶ In addition to contributing to viral infection, SARS-CoV-2 interaction with SR-B1 may also influence cholesterol metabolism. Moreover, some of the patients who survive COVID-19 will present with long-lasting cardiovascular and neurovascular damage with the risk of progressively developing vascular disease, heart failure, and neurovascular cognitive impairment. Consequently, because of the need to engage and collaborate with other researchers and Institutes with similar goals, a Call to Action was issued to investigate RNA biomarkers in SARS-CoV-2-infected patients.¹⁷

2. Vascular mechanics

Advances in biomedical science are often coupled to the development of new technologies and 2020 has seen several examples where novel engineering and physics methods have not only transformed our understanding of disease processes but also provided a basis for treatment decisions. There is intense research on whether measurement of local mechanical forces can predict atherosclerotic plaques at a high risk of destabilization because this would allow pre-emptive treatment to prevent acute coronary syndrome.^{18,19} The past year has witnessed several breakthroughs in this field including clinical observations showing that heterogeneity of structural stress (stress within plaque)²⁰ and sharp gradients in shear stress (flow-induced frictional drag on lumen)²¹ can predict plaque rupture, and a study in pigs demonstrating that variations in shear stress direction can predict plaque growth and disease progression²² (Figure 1). These observations suggest that quantifying localized mechanics may be useful in identifying plaques at risk of destabilization, however there are several obstacles in translating such observations to the clinic including the requirement for specialized imaging equipment and long computing times. However, Morris et al.²³ developed a novel method that analyses clinically relevant intracoronary haemodynamics rapidly. Their virtuQ system computes absolute coronary flow, stenosis and microvascular resistances and flow reserve from routine angiography and pressure wire data, providing a uniquely, comprehensive assessment of the entire coronary circulation. Not only does this advance patient assessment in the catheter laboratory but because the computational fluid dynamics (CFD) method is capable of analysing wall shear stress, this may also provide a research link between biology and vascular anatomy and physiology. The Ebong laboratory also progressed this field by demonstrating that sites of disturbed flow can be targeted

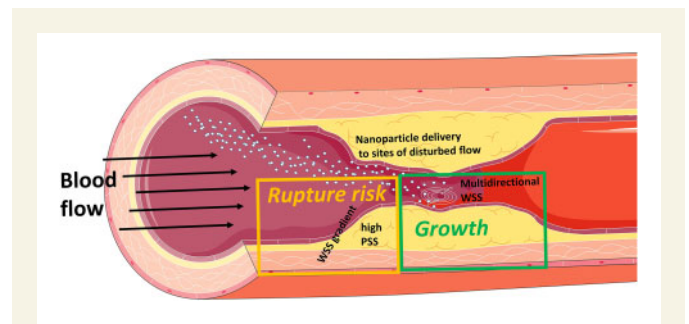


Figure 1 A snapshot of advances in vascular mechanics. Publications in 2020 have linked high plaque structural stress (PSS) and sharp gradients in wall shear stress (WSS) to plaque rupture, and linked multidirectional WSS to plaque growth. Regions of disturbed flow (e.g. multidirectional WSS) can be targeted preferentially with nanoparticles, suggesting a novel therapeutic approach to treat dysfunctional regions of the vasculature. Created with Servier Medical Arts.

preferentially using nanoparticles,²⁴ paving the way for drug delivery to sites of disease progression (Figure 1).

The mechanisms that blood vessels use to sense mechanical forces and convert this information into vascular biological changes have remained largely obscure. This is partly because such mechanobiology experiments require an interdisciplinary approach with input from engineers and physicists to precisely define the mechanical environment and analyse its effects on cellular physiology. Such challenges were overcome this year by the Tzima lab who used a suite of mechanobiology techniques including magnetic tweezers to identify plexin D1 as a mechanoreceptor that allows EC to sense and respond to shear stress.²⁵ Looking downstream from mechanoreceptors, endothelial-to-mesenchymal transition (EndMT) has been recognized as a key pathogenic downstream from disturbed flow.^{26–28} A recent paper found that transforming growth factor (TGF) signalling and EndMT is regulated by histone demethylation,²⁹ suggesting a potential new level for therapeutic targeting of atherosclerosis progression. Despite these advances, further work is needed to elucidate the mechanisms for sensing of mechanical force and to find drugs that can perturb mechanosensitive pathways to reduce disease progression.

At the level of vascular smooth muscle cells (VSMCs) Sanyour et al.³⁰ found a novel link between metabolism and mechanics by showing that cell membrane cholesterol can regulate VSMC stiffness and actin organization. Moreover, analysis of the biomechanical integrity of the aorta has provided insight into optimal medical therapy for patients with a rare autosomal-dominant connective tissue disorder.³¹ Novel engineering approaches were also used to generate endothelialized arteriole-scale blood vessels³² and organoids³³ that might be used to test new therapeutics. Palikuqi and colleagues³³ achieved this by transient reactivation of E twenty-six (ETS) variant transcription factor 2 (ETV2), that is normally restricted to embryonic development of the vascular endothelium, in mature human endothelial cells. Such reactivation of ETV2 in mature endothelial cells led to the formation of perfusable branching, three-dimensional vascular networks in a matrix containing laminin, entactin, and collagen type IV. When implanted into mice these endothelial cells, denominated reset vascular endothelial cells (R-VEC) self-organize into functional blood vessels coated by pericytes, which connect to the host's circulation. When such endothelial cells were introduced into three-dimensional organoids, the formed blood vessels communicated with other cells types present. As a proof of principle, the

authors were able to show, that R-VEC were able to colonize human pancreatic islets and human colon organoids.³³ By using human cells, artificial vessels and organs can be used to analyse the influence of specific genotypes on disease mechanisms, to develop personalized treatments and to reduce the use of animals in cardiovascular research.

3. NETs and other structures

Described first by Brinkmann *et al.*³⁴ in 2004, neutrophil extracellular traps (NETs) were originally thought to be a defence mechanism used by neutrophils against bacterial infections. However, over the last years evidence has accumulated that NETs also play a critical role in a variety of pathophysiological processes in cardiovascular biology such as thrombus formation and inflammation that are crucial events in cardiovascular disease in general and in atherosclerosis in particular. This year there have been several landmark studies that firmly place NETs as a central structure in the pathogenesis of cardiovascular disease.

Employing a murine model of ischaemic stroke Kang and colleagues could show that NETs impair revascularization and blood vessel remodelling after ischaemic stroke.³⁵ In particular the researchers showed an accumulation of neutrophils and consequently also of NETs in the brain following the initiation of ischaemic stroke. When the formation of NETs was abolished by depletion of neutrophils, neovascularization in the brain area affected by stroke was observed. Conversely overexpression of peptidylarginine deiminase 4, which is an essential enzyme for the formation of NETs, reduced this neovascularization (*Figure 2A*).³⁵ Silvestre-Roig and coworkers³⁶ presented evidence that NETs could also indirectly contribute to vascular damage through externalized histone 4 in atherosclerosis. The authors used various mouse models to show that activated smooth muscle cells in atherosclerotic lesions attract neutrophils and induce the release of NETs by these cells through the production of the chemokine CC chemokine ligand 7 (CCL-7). The researchers demonstrated that such NETs induced lytic cell death of smooth muscle cells in atherosclerotic lesions and identified histone 4 as responsible component of NETs for this observation as an inhibitor of histone 4, namely histone inhibitory peptide, prevented NETs-induced cell death of smooth muscle cells. The authors suggest, that neutrophils through histone 4 contained in NETs contribute to the destabilization of atherosclerotic plaques through the depletion of smooth muscle cells in these lesions and that specific inhibition of histone 4 might be a therapeutic strategy without compromising other pathophysiological functions of NETs (*Figure 2B*).

It is well established that cardiovascular events show a circadian pattern.³⁷ In that respect, a study performed by Adrover and colleagues³⁸ is of particular interest. They could show that the granule content of neutrophils and subsequently their ability to form NETs undergo circadian changes. Using mouse models of acute lung injury, the researchers identified the receptor C-X-C chemokine receptor 2 (CXCR2) and a specific modulator of the circadian rhythm, namely brain and muscle ARNT-like 1 (Bmal1), being responsible for a circadian pattern of susceptibility to inflammatory lung injury. Furthermore, Adrover and coworkers³⁸ showed a striking circadian association of the severity of pneumonia and pneumonia-related deaths in humans to the granule content of neutrophils and to their ability to extrude NETs. The authors conclude that their findings might help to explain an observed circadian pattern in disease severity and events seen in other pathologies driven by inflammation (*Figure 2C*).

In contrast to detrimental effects of NETs described above, a recent paper by Binet *et al.*³⁹ provides evidence for a beneficial role of NETs in retinopathy. In a mouse model of ischaemic retinopathy, the researchers found that during vascular remodelling, senescent endothelial cells attracted neutrophils and triggered the extrusion of NETs by these cells. These NETs proved to be instrumental for effective and beneficial vascular remodelling by inducing apoptosis in senescent vascular cells and by removing pathological retinal vessels. The authors conclude that NETosis triggered by senescent vasculature might not only contribute to beneficial vascular remodelling as seen in their model of retinopathy but might also be involved in NETs-induced thrombosis seen in stroke and myocardial infarction in elderly individuals (*Figure 2D*).

Recent evidence has also implicated NETs in the pathogenesis of severe COVID-19 infections. Radermecker and colleagues⁴⁰ found NETs not only in the lung airways but also in the pulmonary interstitial tissue and in the vasculature of the lung in post-mortem tissue obtained from patients who died from COVID-19.⁴⁰ In the airways, NETs were associated with fibrin pointing towards leakage of plasma proteins caused by thrombotic occlusion of blood vessels. Consistent with that observation, NETs in the lung vasculature were seen in microthrombi of arterioles. The authors suggest that NETs might represent a therapeutic target in an effort to prevent tissue damage and restore blood flow also in patients suffering from COVID-19 infections (*Figure 2E*).

4. Novel therapeutic approaches and targets

The year 2020 brought also some new developments in the identification of new therapeutic targets in vascular biology as well as in the development of new therapeutic approaches to combat vascular pathologies. Small molecules were designed to interfere with disease development, novel cell-based therapies were employed and the therapeutic potential of microRNAs was exploited.

O'Meara and colleagues⁴¹ tested the ability of a variety of small polyanions (SPAs) to counteract detrimental effects of free extracellular histones or extracellular histones bound to NETs. They identified such SPAs that were able to block the histone-induced damage of ECs and the platelet activation and deformability of red blood cells caused by histones. SPAs exerted these effects by preventing the disruption of the cell membrane lipid bilayer through histones. Furthermore, the authors could show in various animal models, such as a sepsis model or models of ischaemic reperfusion and deep vein thrombosis, that the protecting effect of SPAs against histone-induced damage was also operative in the *in vivo* setting. Finally, the authors showed that SPAs inhibited also the injury of ECs when these cells were incubated with sera from patients suffering from sepsis or myocardial infarction that were rich in extracellular histones. With their work, the authors have identified SPAs as promising therapeutic agents to counteract the cytotoxic effect of extracellular histones seen in various pathologies characterized with wide-spread tissue damage of NETs extrusion (*Figure 2F*).⁴¹

Kontos and co-workers⁴² designed a small CXCR4-mimic that acts as a decoy to bind macrophage inhibitor factor (MIF) thereby preventing the inflammatory action of MIF that plays a key role in the pathogenesis of atherosclerosis. The selected peptide binds to a short amino acid sequence in the extracellular loop 1 of MIF and was designated MIF-specific human CXCR4 mimic-ECL1 (msR4-L1). Importantly, msR4-L1 selectively binds to MIF and does not interfere with binding of CXC ligand 12 (CXCL12) to CXCR4 or CD74 binding to MIF and thus does not block

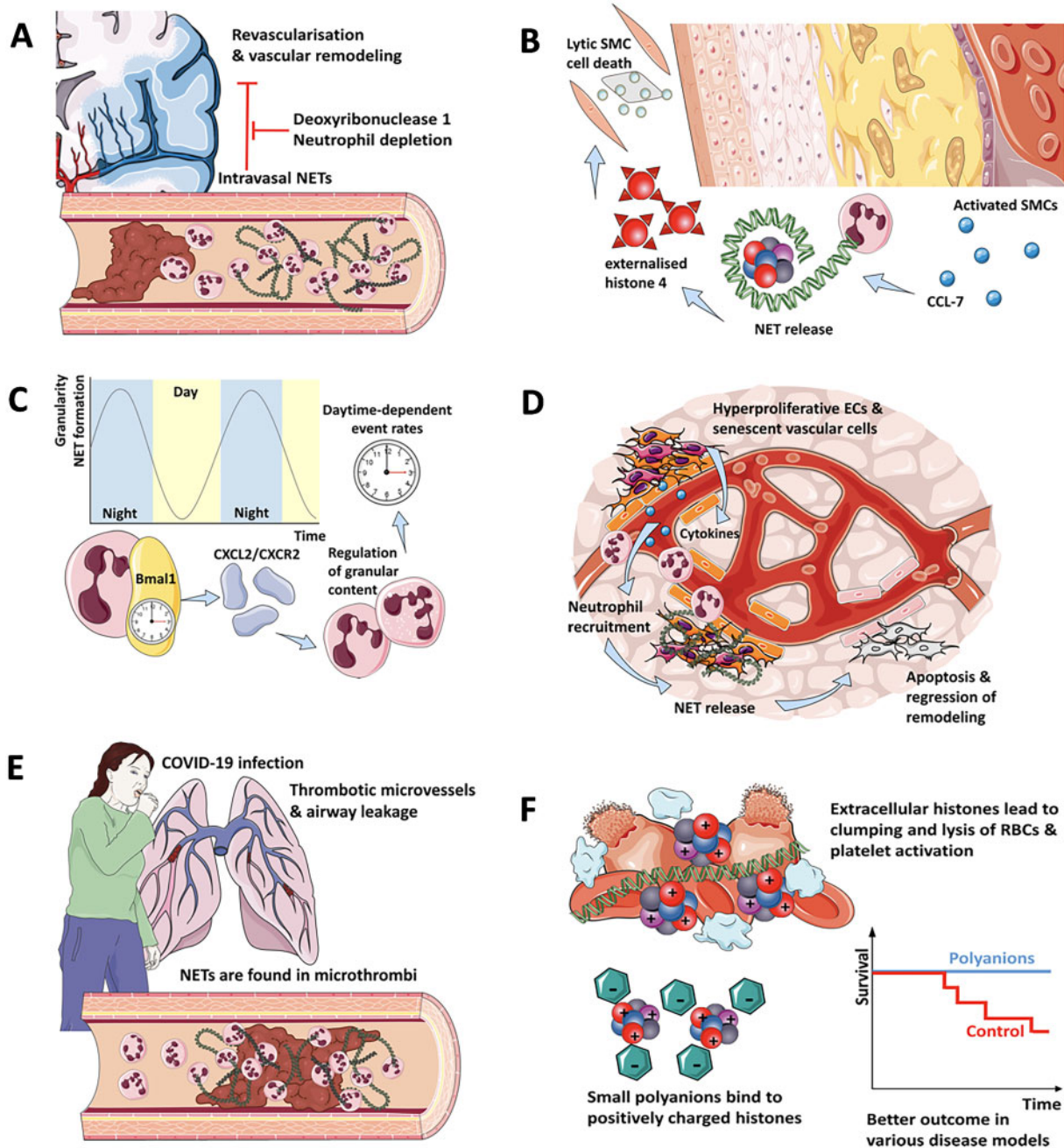


Figure 2 NETs and vascular biology. Publications in 2020 found that NETs influence (A) ischaemic stroke,³⁵ (B) SMCs in atherosclerosis,³⁶ (C) circadian rhythm,³⁸ (D) senescent vascular cells,³⁹ (E) COVID-19 infection⁴⁰ and they can be inhibited using small polyanions⁴¹ (F). Abbreviations: CCL-7, CC chemokine ligand 7; COVID-19, coronavirus disease 2019; CXCL2, C-X-C chemokine ligand 2; CXCR2, C-X-C chemokine receptor 2; ECs, endothelial cells; NETs, neutrophil extracellular traps; RBCs, red blood cells; SMCs, smooth muscle cells. Created with Servier Medical Arts.

the respective athero- and cardioprotective effects of neither CXCL12 nor CD74. Using various cell culture models and mouse models of atherosclerosis, the researchers could show that the peptide blocked the atherogenic activities of MIF *in vitro* as well as *in vivo*. Finally, using tissue samples from human atherosclerotic plaques, they provide evidence, that the designed peptide binds to MIF *in situ*. Thus, the researchers have developed a small, highly specific peptide that has therapeutic potential to specifically counteract the detrimental effects of MIF in the pathogenesis of atherosclerosis.⁴²

The glucocorticoid-induced tumour necrosis factor receptor family-related protein (GITR) was identified by Shami and colleagues⁴³ as yet another driver of destabilization of atherosclerotic plaques and as such as yet another potential target to prevent plaque rupture. The authors found elevated levels of GITR in atherosclerotic plaques obtained from symptomatic patients compared to plaque tissue from asymptomatic individuals. GITR was predominantly present in macrophages in the shoulder regions and in the base of the lesion but not in the fibrous caps. The authors created mice double-deficient in GITR and ApoE and

showed that in these mice plaque size was smaller as opposed to ApoE deficient mice and expressed a more stable phenotype with a thicker fibrous cap, a smaller necrotic core and less macrophages present. On further analysis, the authors showed that macrophages from G1TR deficient mice exhibited decreased mitochondrial activation and a less inflammatory phenotype. *Another study found that LDLR-related protein 5 (LRP5) participates in lipid uptake by macrophages and contributes to proprotein convertase subtilisin kexin 9 (PCSK9) transport to the plasma membrane and lipid-induced inflammation through the toll like receptor 4 (TLR4)-nuclear factor κ B (NF κ B) pathway.*⁴⁴ These results offer new targets to prevent the progression of atherosclerosis and the increased risk of cardiovascular events.^{43,44}

The year 2020 has seen notable discoveries in microRNAs (miRs) biology including first-in-human studies of two cardiovascular anti-miRs.^{45,46} Particular miRs have been identified as potentially therapeutically relevant in pulmonary hypertension in a paper published by Sindi and coworkers.⁴⁷ The researchers studied the profile of exosomal microRNAs induced in human pulmonary artery ECs by overexpression of Krüppel-like factor 2 (KLF2) in these cells. They showed that exosomes of such ECs protected cells from apoptosis and reduced the expression of the transcription factor NF κ B that is a key modulator for inflammatory activation. On further analysis of the content of these exosomes, the scientists identified miR-181a-5p and miR-324-5p as being responsible for these effects. By pathway analysis, they showed that miR-181 and miR-324 expression was associated with downregulation of inflammatory and angiogenic pathways. Finally, as a proof of principle *in vivo*, the authors present evidence that application of miR-181 and miR-324 in a mouse model of pulmonary hypertension reduced right ventricular systolic pressure and right ventricular hypertrophy in such mice as compared to control animals. Furthermore, modulators of inflammation and angiogenesis in the lungs of mice treated with these microRNAs were also reduced.

Of note, Santovito *et al.*⁴⁸ identified a novel function of endogenous miRNAs outside of the RNA-induced silencing complex (RISC) through exploiting a direct aptamer-like interaction between miR-126-5p and the effector protease caspase-3, a mediator of apoptotic cell death. In detail, under conditions of endothelial autophagy, miR-126-5p specifically interacts with the RNA-binding protein Mex3a in low-molecular-weight RISCs and shuttles into the nucleus, where it dissociates from Argonaute-2 (AGO2) and binds caspase 3 to prevent dimerization and proper active site formation. This non-canonical protein: RNA interaction inhibits the proteolytic activity of caspase-3 and limits EC apoptosis. These papers provide further evidence for the therapeutic potential of particular microRNAs in vascular medicine.^{47,48}

The implantation of vascular grafts has become a wide-spread revascularization therapy to restore blood flow in ischaemic tissue. Efficient endothelialization of vascular grafts, however, remains difficult. Smith and colleagues⁴⁹ present a new approach to tackle this problem by engineering blood vessels using small intestine submucosa (SIS) as a scaffold. They showed that such SIS with immobilized heparin and vascular endothelial growth factor (VEGF) were endothelialized in a large animal ovine model *in vivo*. On further analysis, the scientists discovered that surprisingly the vast majority of cells adhering to the matrix were not of endothelial lineage but rather circulating monocytic cells. In *in vitro* experiments, they could show that these monocytes bind to VEGF immobilized to SIS. Over time, these monocytes undergo differentiation into macrophages resembling an M2 phenotype and finally into ECs. This differentiation process was monitored by following the expression of surface markers characteristic for the respective cell type. Most surprisingly, mature monocytes, which do not proliferate in the circulation,

started to proliferate shortly after adhering to SIS. Based on their results, the authors have established a protocol for seeding of monocytes onto SIS vascular grafts and differentiation of these monocytes via M2 macrophages into ECs. This procedure might offer new ways to solve the current problems related to endothelialization of vascular grafts.⁴⁹

5. Big data, smartphones, and other wearable devices

Recent advances in computer modelling, machine-learning, and sequencing technology have made significant impact on biomedical research. These developments have made possible the analysis of large data sets resulting in major breakthroughs in vascular biology.

The advent of smartphones and wearable devices has provided researchers with the opportunity to collect vast amounts of biomedical data from patients that can be used for risk prediction and for monitoring the efficacy of therapies. Using such an approach, Avram *et al.*⁵⁰ used vascular signals as a digital biomarker for diabetes. In particular, the researchers used data from photoplethysmography recorded via regular smartphones from individuals participating in this study to develop a deep neural network (DNN) to detect prevalent diabetes in a primary cohort, in a validation cohort and in a prospective clinical cohort. The DNN score obtained was predictive of diabetes independent of age, gender, ethnicity, and body mass index. On further analysis, the researchers showed a positive association between this score and HbA1c. This latter observation led the scientists to hypothesize that this smartphone-based biomarker might not only be used to predict diabetes but also to monitor disease severity and efficacy of therapy.⁵⁰

Rwei and co-workers⁵¹ developed a wearable device for monitoring cerebral haemodynamic parameters in paediatric patients. The device is small, wireless, and runs on batteries. Its flexible design allows for easy application on and removal from heads of paediatric patients without skin irritation or injury. Using photoplethysmography and near-infrared spectroscopy, the device monitors systemic and cerebral haemodynamic parameters such as heart rate, cerebral and peripheral oxygenation and cerebral pulse pressure and vascular tone. In their paper, the researchers also present clinical pilot data obtained from patients with and without congenital central hypoventilation syndrome, which have indicated the feasibility of the device in the clinical setting. The authors conclude that their biosensor might improve paediatric care in patients at risk of cerebral damage due to impaired cerebral haemodynamics.⁵¹

Bai and colleagues⁵² performed a population-based phenome-wide association study (Phe-WAS) on cardiac and aortic structure and function by analysing cardiovascular magnetic resonance images from almost 27 000 individuals enrolled in the population-based UK biobank with a machine-learning-based pipeline. Using the data obtained, the authors tested possible associations of quantitative imaging phenotypes of six anatomical structures with non-imaging phenotypes such as, e.g. cardiovascular risk factors, demographics, anthropometrics, and life style parameters. They found such associations between imaging phenotypes and systolic blood pressure, smoking, alcohol consumption, physical activity and diabetes, all of which are known to impact on risk to develop cardiovascular disease. Rather unexpected associations were found between birth weight, mental health, and cognitive performance and the imaging phenotypes. On the basis of these findings, the authors emphasize the future potential of image-based biomarkers.⁵²

In a genome wide association study (GWAS) comparing 5236 control subjects with 270 individuals suffering from spontaneous coronary artery

dissection (SCAD), Saw and colleagues⁵³ described an association of rs12740679 at chromosome 1q21.2 with this pathology implicating the gene regulating the expression of the matrix protein A disintegrin and metalloproteinase with thrombospondin motifs-like protein 4 (ADAMTSL4) and lower expression levels of ADAMTSL4 with higher risk of SCAD. Furthermore, the authors also confirmed a respective association of SCAD with variants in the genes for phosphatase and actin regulator 1 (PHACTR1) on chromosome 6p24.1 and the low density lipoprotein receptor-related protein 1 (LRP1) gene at chromosome 12q13.3 as well as for the gene for multidrug resistance protein 6 (MRP6)/KCNE3 on chromosome 21q22.11. Analysis of a polygenic risk score revealed a higher risk for SCAD in patients suffering from fibromuscular dysplasia and a significantly lower risk for atherosclerosis and myocardial infarction in patients at risk for SCAD. The authors hypothesize that this inverse relationship and opposing genetic risk pattern for SCAD-associated myocardial infarction on the one hand and myocardial infarction caused by atherosclerosis of coronary arteries on the other hand points towards particular vascular genotypes impacting on the pathogenesis of different types of myocardial infarction.⁵³

In another GWAS, Klarin *et al.*⁵⁴ compared 7642 cases of abdominal aortic aneurysm (AAA) with 172 172 controls and identified 14 new loci associated with AAA in addition to 10 already known loci. The authors describe a causal link between AAA and a genetically caused increase in diastolic blood pressure of 10 mm Hg. No genetic link was found with increased systolic blood pressure. Most of the identified risk variants for AAA were linked to aneurysms in at least one vascular territory examined. A polygenic risk score incorporating 29 of the variants showed a significant association with AAA independent of family history and smoking. The authors conclude that their polygenic risk score might improve current screening procedures.⁵⁴

In a meta-analysis from 1.3 million individuals, Surendran and co-workers⁵⁵ identified 87 rare variants associated with blood pressure regulation. Thirty-two of these variants were located in genes with known implication in blood pressure control in mouse models and in monogenic disorders. In addition, the researchers described 106 new genomic regions associated with blood pressure. Interestingly, single nucleotide variants related to systolic blood pressure were found more often in regions of open chromatin in foetal as compared to adult myocardial tissue, suggesting that these variants might impact on cardiac foetal development. Furthermore, only two of the 106 newly described loci were significantly associated with four blood pressure traits analysed, namely hypertension, inverse-normal transformed systolic blood pressure, diastolic blood pressure and pulse pressure. Unexpectedly, none of these new variants was associated with hypertension only. The authors emphasize the utility of rare variant analyses to identify new candidate genes for blood pressure regulation.⁵⁵

Another genome wide meta-analysis performed by Guo and colleagues⁵⁶ analysed 59 674 cases of migraine and 316 078 control cases. In addition, the researchers analysed combined statistics for blood pressure meta-analysis in 757 601 individuals. Three blood pressure traits were included in the study, namely systolic blood pressure, diastolic blood pressure, and pulse pressure. The authors describe the strongest positive genetic correlation between diastolic blood pressure and migraine. Five loci coming up in transcriptome wide association studies (TWAS) as well as in SNP-analysis shared involvement in both the pathobiology of migraine and the regulation of blood pressure such as vascular development, endothelial function, inflammation, and calcium homeostasis. In particular, the authors of the study point towards the gene responsible for the expression of the $\alpha 2B$ adrenergic receptor (ADRB2), which is a receptor for a variety of neurotransmitters regulating vascular tone and

which in addition is thought to be involved in the pathogenesis of migraine. The authors note that in contrast to their findings concerning migraine, the association of cardiovascular disease with blood pressure seems to be caused by systolic blood pressure and that therefore the observed association of migraine and cardiovascular disease might be caused by different mechanisms of blood pressure regulation.⁵⁷

6. Single cell omics and cell plasticity

The advent of single cell omics technologies is revolutionizing biomedicine by mapping out the cellular basis of disease in unprecedented detail. While single cell RNAseq (scRNAseq) studies were previously the preserve of specialized molecular biology labs, the past 12 months has seen this technology move towards mainstream use with important breakthroughs in the cardiovascular field.

Previous studies of murine atherosclerotic plaques have revealed significant phenotypic diversity in leukocytes. Although some phenotypes are conserved in multiple studies, others have not been observed consistently. There are several possible explanations for divergence between studies including differences in sample preparation and other methodological aspects including bioinformatics. Comparison between publications is also complicated by differences in nomenclature used to describe cell subsets. In an attempt to unify some of these observations, Zernecke *et al.*⁵⁸ performed a meta-analysis of data from mouse aortic plaques and found five macrophage subsets (including one novel type) and identified prominent and separate populations of monocytes, neutrophils, dendritic cells, natural killer cells, innate lymphoid cells, and T cells. These data emphasize the importance of data sharing between groups to strengthen observations, which is particularly important when analysing rare cell subtypes. The diversity of EC phenotypes has been illuminated by scRNAseq generation of an atlas of murine EC⁵⁹ and by integrating scRNAseq and transcriptome data in multiple tissues.⁶⁰ Both of these studies revealed that EC phenotypes vary considerably according to their tissue of origin, emphasizing their bespoke physiological and metabolic functions.

Over the past 12 months, the scRNAseq field has progressed with more studies of human tissues including the heart.⁶¹ For example, Fernandez *et al.*⁶² used CITE-SEQ (which brings together proteomic and transcriptomic data) to analyse carotid artery plaques and revealed specific subsets of CD4+ T cells and macrophages that were specifically associated with symptomatic disease but not observed in plaques from asymptomatic patients. These data suggest that specific leukocyte subsets may be a biomarker of symptomatic disease and they also prompt further investigation of the role of these subsets in pathology. This concept was also suggested by Depuydt *et al.*⁶³ who found 14 subsets of cells in human carotid artery plaques including lymphocytes, macrophages, endothelial cells, smooth muscle cells, and mast cells with considerable interconversions between these 'ground level' states including evidence of endothelial-to-mesenchymal transition. In another study, scRNAseq of human carotid endarterectomy specimens coupled to lineage tracing in murine models demonstrated considerable SMC plasticity in late-stage atherosclerosis including transition to pathogenic osteogenic cells.⁶⁴ Collectively, these papers have confirmed that cellular phenotypic diversity and cellular interconversions occur in real-world clinical scenarios and they are not merely the preserve of inbred transgenic mice.

In the past 12 months, scRNAseq-based studies also found further evidence of endothelial plasticity during development,^{65–67} and revealed novel mechanisms of vascular remodelling in hypertension⁶⁸ and

ageing.^{69,70} Indeed, cell plasticity provides new opportunities for therapeutic targeting as shown this year by Artiach *et al.*⁷¹ who found that omega-3 polyunsaturated fatty acids can protect against aortic valve stenosis by activating a pathway involved in smooth muscle cell phenotypic switching,⁷² and the elucidation of oligomeric S100A4-TLR4-NFκB pathway driving VSMC phenotypic transition in atherosclerosis.⁷³ A limitation of scRNAseq is the lack of spatial information since tissues are digested into a single cell soup. Therefore, perhaps one of the most exciting techniques to emerge in recent years is spatial transcriptomics in which scRNAseq data is assigned to individual cells with retention of normal tissue architecture. This approach was applied to cardiovascular science by Asp *et al.*⁷⁴ who integrated scRNAseq data with spatial transcriptomic information within tissue slices of the developing human heart. The next few years are likely to see spatial transcriptomics applied to atherosclerosis and other vascular diseases to generate unprecedented molecular details of their pathogenesis.

There have also been major advances in our understanding of metabolic plasticity in the vasculature. While the contribution of glycolysis to angiogenesis is well accepted, the role of mitochondrial respiration has been controversial. This was addressed by the Kashkar lab who showed by EC-specific genetic deletion that the mitochondrial respiratory chain is critical for neoangiogenesis in adult mice.⁷⁵ This observation has relevance for therapeutic control of angiogenesis in tissue repair and cancer. Other key studies coupled metabolism to the regulation of EC barrier function,^{76,77} linked exercise with NADPH oxidase 4 (NOX4)-dependent endothelial function,⁷⁸ and found catabolism of adenosine driving VSMC proliferation.⁷⁹ EC junctions are highly dynamic structures that open and re-anneal in response to specific signals and are disrupted in many vascular diseases. Montagne *et al.*⁷⁶ found that dysfunction of EC junctions (blood–brain-barrier; BBB) in Alzheimer's disease is associated with specific allelic forms of apolipoprotein E (ApoE), thereby providing a mechanism linking cholesterol transport with BBB integrity. In addition, Cheung *et al.*⁷⁷ demonstrated that EC junction dynamics are also controlled by glycolysis which drives the reannealing process, suggesting a novel mechanism for therapeutic control of endothelial barrier function.

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