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Title: Perivascular Spaces in the Brain: Anatomy, Physiology, and Contributions to Pathology of Brain Diseases.

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Abstract

Perivascular spaces (PVS) represent a range of passageways around arterioles, capillaries and venules in the brain along which a range of substances can move. Roles for PVS in interstitial fluid drainage and immunological protection have been known for decades. However, PVS have come to prominence recently through potential roles in brain interstitial fluid and waste clearance particularly during sleep, and in the pathogenesis of small vessel disease, Alzheimer's disease and other neurodegenerative and inflammatory disorders. Recent advances have enabled in vivo studies of PVS function in intact rodent models while awake or asleep, of human PVS morphology related to cognition, vascular risk factors, vascular and neurodegenerative brain lesions, sleep patterns and with detailed cerebral haemodynamics. Although research on the PVS is longstanding, many questions remain. What is clear is that normal PVS function is important for maintaining brain health. Notions that PVS are 'curiosities' on neuroimaging, or artefacts on pathology, may have delayed scientific progress. Several tools are now available to advance understanding and clinical awareness of PVS in the context of vascular, inflammatory and neurodegenerative diseases. Knowledge of PVS is relevant to clinicians in neurology, psychiatry, geriatric and general medicine, vascular specialists, and radiologists. Here, we review PVS anatomy, physiology and pathology, translating from models to humans, highlighting knowns, unknowns, controversies, and clinical relevance.

Introduction

The small spaces that surround small blood vessels as they pass through the brain are variously known as perivascular or paravascular spaces (PVS), including periarteriolar, pericapillary and perivenular spaces. Although these spaces were described on pathology of the human brain over a century ago, they have come to prominence in the last decade with advances in sensitivity of in vivo visualisation tools, such as magnetic resonance imaging (MRI) in humans or 2-photon imaging (2PI) via cranial windows in rodent models. These modalities are enabling opportunities to understand the physiology, importance and complex nature of the brain's fluid and waste clearance systems. Such fundamental aspects of the 'dynamic brain' underscore how PVS may influence the pathogenesis of common cerebrovascular, neuroinflammatory and neurodegenerative disorders.

Why now?

Converging information from human studies and rodent models suggests a role for normal PVS function in maintaining brain health, and of PVS dysfunction in common neurological disorders (Box 1). Human MRI studies show that PVS visibility and size on MRI increase with ageing,[1-4] in association with some vascular risk factors,[1,5] with MRI features and clinical features of small vessel disease,[6,7] in Alzheimer's disease (AD),[3,8,9] in multiple sclerosis (MS),[10] and sleep disorders.[11] Rodent models demonstrate that fluid flow through the PVS and exchange with interstitial fluid (ISF) increases during sleep when compared to wakefulness,[12] providing one explanation for the physiological importance of sleep to brain health. They also show that fluid transport within arteriolar PVS is impaired when blood pressure (BP) is elevated,[13,14] and in AD models with aggregation of amyloid-β_{1-42} protein. In rodent models and patients with cerebral amyloid angiopathy (CAA), there is aggregation of amyloid-β_{1-40} in the PVS around arterioles indicating failure of clearance.[15-18] Additionally, MRI-visible PVS in people are highly heritable, like other small vessel disease (SVD) lesions such as white matter hyperintensities (WMH).[19]

Many longstanding controversies

On the other hand, there has been controversy about many aspects of PVS since their earliest descriptions in the mid-1800s (summarised in[20]), and they remain controversial (Box 2).[21,22] There is current debate about their associations with vascular risk factors, neurological diseases, or with features of SVD particularly white matter hyperintensities, (WMH),[1,5] or whether PVS are an epiphenomenon. They have been considered as histopathological fixation artefact, or due to brain tissue

loss during aging, and thus overlooked. There is debate about their anatomical structure,[23] relationships to arterioles, capillaries and venules,[24] the direction of ISF and solute drainage out of the brain, connections with cerebrospinal fluid (CSF) compartments,[25] role of aquaporin 4 (AQP4),[21] relationships to meningeal lymphatic drainage channels,[26-28] and role during sleep.[12,29] These unresolved issues may reflect differences in the populations studied, different regions of the brain, accounting for related variables (e.g. age, risk factors), different models, or methodologies.

Aims of the review

In this review, we aim to describe and discuss knowns and unknowns about PVS, their anatomy, physiology and role in pathology, focusing on their normal function and what goes wrong in neurological disease. Here, the 'perivascular space' is defined broadly as including the small spaces that are visible in the brain on MRI or at post-mortem running into the brain with direction consistent with that of perforating vessels, and are thought to be contiguous with the pericapillary *potential* spaces.[30] Since their dynamics are so important, we focus on in-vivo data and recent methods enabling more detailed in vivo analysis of PVS.[31,32] Importantly, accurate in vivo assessment of PVS, static[32] and dynamic,[33] may provide useful biomarkers and novel therapeutic targets,[34] at early stages in disease development when future interventions may be most successful. Following a brief summary of PVS history to orientate the reader, we focus on 1) relevant clinical evidence for the importance of PVS as seen on MRI in human health and disease, and supporting data from histopathology where available (Box 1); 2) information from preclinical studies on PVS structure and function in health and disease models; while highlighting 3) major controversies and gaps in knowledge (Box 2).

A brief, but relevant, history

PVS were described originally in 1849 by Pestalozzi, but are often ascribed to Rudolf Virchow (German pathologist 1821-1902) and Charles Robin (French anatomist, 1821-1885), who described spaces around brain perforating vessels on histopathology in 1851 and 1859 respectively.[20] Despite PVS being referred to ever since as 'Virchow-Robin spaces', these two experts disagreed on a) whether or not PVS connected with the subarachnoid space, and b) whether or not PVS were a type of 'brain lymphatic', Robin's theory that PVS connected with perineuronal spaces,[20] is now recognised as lymphatics.[8,17,25] Enlargement of periarteriolar PVS at post-mortem was described by Durand Fardel (1843) who with others referred to their

appearance in basal ganglia as ‘etat crible’.[35] PVS enlargement was noted to be pathological, accompanied by perivascular inflammatory cell infiltration and arteriolar morphologies consistent with the arteriolosclerosis and fibrinoid necrosis described in the 1950’s.[36]

Some original concepts of PVS *function* were derived from in vivo experiments in rodents conducted in the *early 1900s*. These were actually aimed at determining how CSF was produced and its circulation, and showed that Prussian blue, injected into the subarachnoid space followed by sacrifice after various time intervals, entered the PVS.[37,38] Multiple experiments in the 1920s[39] suggested that PVS extended along arterioles, capillaries and venules, communicated freely with perineuronal spaces and other potential spaces between glial elements and fibre tracks. This interpretation was based on dyes injected into CSF simultaneous with intravenous hypertonic saline that increased dye uptake but also caused tissue shrinkage and, by not representing a physiological state, may have contributed to ideas about fixation artefacts. However, the use of hypertonic saline itself is interesting, since the ability to increase CSF uptake into PVS deliberately might offer routes to deliver new therapies in several neurological disorders ([29,34,40,41] and summarised in[8]).

Later experiments, aiming to sort out controversies,[20] compared injecting Indian ink into the live adult rat subarachnoid space with simultaneous intravenous (iv) hypertonic saline, and in parallel experiments in newborn rats, tiny amounts of colloidal carbon were injected through the parieto-occipital suture (after first removing tiny amounts of CSF to avoid non-physiological pressure increases) daily for three weeks before sacrifice. The Indian ink penetrated consistently into the basal ganglia PVS but variably in other areas, and carbon particles were seen around arterioles in between the arteriole outer wall and a membrane formed from, and contiguous with, pia mater. A space seen external to the pial membrane was considered to be artefact from tissue shrinkage since it only occurred with use of hypertonic saline,[20] and may be a source of the persisting idea today that many histological and MRI-visible PVS are artefact.

With the advent of widespread use of human MRI in the 1980s, small linear fluid-filled structures were visible running in parallel with the known direction of the perforating vessels in the midbrain, hippocampus, basal ganglia and cerebral hemispheric white matter of the centrum semiovale.[42] These are more visible on heavily T2-weighted than T1-weighted MRI sequences, since the bright white fluid signal on T2 is

highlighted against the dark brain tissue background.[23,43] Initially, these were largely ignored until the early 2000s when several groups noted that PVS visibility varied widely and therefore began to study their clinical phenotype and risk factor associations in more detail.[9,23,44-46]

Since then, major advances in in vivo experimental methods such as 2-PI via cranial windows[47] in alert animals,[12] dynamic MRI imaging in rodents tracking Gd injected into the cisterna magna[48] and modelled mathematically using optimum mass transport approaches,[33,49] advances in analysis of microscopic and MRI images,[50] and more sophisticated histopathological and electron microscopic techniques, have accelerated research into PVS structure and function. Some of this is now translating to in vivo human MRI methods,[32,51,52] with much more sophisticated image analysis,[13,53] and reliable information from both laboratory and human sources is now converging.

PVS anatomy, as seen on human MRI

Reading historical and recent papers on the histological structure of PVS, and whether or not they connect to which other spaces, is likely to leave one feeling *thoroughly confused*. Therefore, we will start with the PVS that are visible on human brain MRI from routine imaging in the clinic – this much we know (Box 1) - and return to consider the details of the brain's fluid drainage system and pathophysiological implications later.

PVS (in some form, including potential or virtual passageways) are thought to surround arterioles, capillaries and venules as they run through the brain. The PVS that are visible in the brain parenchyma on MRI run perpendicular to the brain's surface and in directions that are parallel to and spatially correlated with, perforating vessels. PVS appear linear if running in the plane of the image and dot-like if running perpendicular to the image (Figure 1).[43] Therefore, it is reasonable to believe that these visible PVS are related to perforating vessels.

Usual locations in the brain

One or two small PVS are often visible on MRI even in the very young brain, but they usually become more visible with increasing age.[2,8] The regions of the brain where they are typically seen, even when few in number (Figure 1), are in the:

a) basal ganglia (lentiform nucleus, internal and external capsule) immediately superior to the basal perforating substance where they are often visible connecting with the cisternal CSF (Figure 2);
b) centrum semiovale, running centripetally from the external aspect of the white matter towards the lateral ventricles, including in the anterior temporal poles in monogenic SVDs such as CADASIL;[54]
c) hippocampus; and
d) midbrain, pons and sometimes in the cerebellar white matter.[6]
As a generalization, individuals with numerous PVS in one region tend to have numerous PVS in all typical areas. For instance, basal ganglia PVS correlate highly with centrum semiovale PVS.[55] However, sometimes PVS can be more prevalent, or larger, in the basal ganglia than in the centrum semiovale, or vice versa, and their associations can differ (see below). Therefore virtually all rating scales devised to date (summarised in[43]) quantify PVS by brain region.

Further justification for separate quantification of PVS by region reflects anatomical differences. On high field (7T) MRI, basal ganglia PVS are seen to communicate directly with the basal subarachnoid cisterns with the inferior end of the PVS fanning out to join the cistern (Figure 2): basal PVS then run superiorly through the basal ganglia, with frequent calibre changes along their tracks, to end around the superior aspect of the basal ganglia.[4] In contrast, the PVS in the centrum semiovale, which surround vessels that enter the brain from the convexity cortex, appear to start a few millimetres deep to the cortex and converge smoothly towards the lateral ventricles, ending a few millimetres to a centimetre from the supero-lateral walls of the ventricles.[4] A similar appearance of the visible PVS starting immediately deep to the cortex was seen in human brain at post-mortem,[56] in vivo on MRI at lower field strengths (Figure 2),[43] and on histology and 2-PI in rodent models (summarised in[8]).

Periarteriolar, perivenular, or both?
There is debate about whether MRI-visible PVS surround arterioles, venules or both,[57-59] and most human MRI at conventional field strengths cannot easily identify perforating arterioles and venules directly. A small study with high field (7T) MRI in humans used the directional effects of flowing blood in the magnetic field to demonstrate that MRI-visible PVS correlated spatially with arterioles not venules (Figure 3).[4] At lower field strengths (1.5 or 3T) and with good quality images, it is possible to see PVS with a T2 sequence and venules with a blood-sensitive susceptibility-weighted sequence (T2*) in the centrum semiovale, the latter

distribution being consistent with known venular anatomy.[60] Combining these sequences seems to suggest that the venules are distinct from the PVS (Figure 3). Of course, these are isolated small samples. It would be imprudent to state that *all* MRI visible PVS are only around arterioles, as such a statement would imply that PVS are not tied to venular dysfunction. Collagenosis of the deep medullary venules was described on histopathology in the periventricular white matter alongside arteriolosclerosis in older patients known to have leukoaraiosis on pre- or post-mortem MRI.[57] Diffuse patchy periventricular WMH correlated with collagenosis of the deep penetrating venules at autopsy in a patient with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalophathy (CADASIL).[59] But the extent to which venous collagenosis contributes to PVS visibility on MRI in sporadic SVDs, in addition to periarteriolar PVS, and how commonly venous collagenosis occurs in monogenic SVDs like CADASIL, is not known and needs investigation.

Finally, methods are now available to visualise venules close to active lesions in multiple sclerosis,[61] where focal PVS dilatation has been observed during active inflammation,[10] making it possible to see if the visible PVS correspond with venules, and if similar abnormal venules are ever visible in vascular WMH or in recent small subcortical infarcts in patients with sporadic or genetic SVDs. So far, in humans, an abnormal arteriole, perhaps thrombosed, has been documented on MRI in the centre of a recent small subcortical infarct, including some where there appeared to be a prominent periarteriolar space,[62] but not venules.

How can PVS be quantified on MRI

Virtually all studies of PVS on MRI and their associations have relied so far on visual scores for quantification since, until recently, computational image analysis methods were not sufficiently advanced to quantify such small structures (Figure 1). Several visual scoring methods have developed over the last 18 years,[44] but all use similar approaches and in general rate PVS in similar brain areas (summarised in [43]). It is too time consuming to manually count individual PVS in a scan slice especially in large studies. Therefore, most scores categorise the severity of PVS based on the approximate number of PVS in an anatomically defined region.[43,46,63] Thus fewer than 10 in the basal ganglia on a defined brain scan slice might have a score of 1, between 11 and 20 a score of 2, 21-40 a score of 3 and >40 a score of 4.[43] These scores are quick and practical to use in clinical research, have good reliability and

repeatability,[43] and have therefore been applied in many individual studies including some totalling several thousand subjects to date.[1] However, qualitative scores are relatively insensitive and have floor and ceiling effects.

With advances in isotropic 3D MRI acquisition and computational image analysis methods, it has become possible to quantify PVS computationally, and several methods are available.[3,32,64] These require further testing but appear promising, with greater sensitivity to change. Some methods can quantify several PVS characteristics in addition to frequency (e.g. total PVS volume, individual PVS size, length, width, sphericity, directionality, proximity to other structures) and assess spatially-correlated measures of tissue integrity.[32] Early studies show high agreement between visual PVS scores and computational PVS counts, volumes and individual size measures (Figure 1).[32]

There is no equivalent PVS quantification yet for human tissue sections, although similar approaches could be applied, and also used to quantify rodent PVS. However, dynamic MRI methods to track CSF tracer uptake into PVS and distribution in rodents are greatly advanced compared to what is currently possible in humans.[33,48] Optimum mass transport (OMT) is a method to model mathematically the transport of one mass to another in a manner that minimizes a given cost function. It has been applied in many areas, but in image processing, 'mass' may be represented by signal intensity and can be applied to quantify an image registration/optical flow technique. OMT gives a natural temporal interpolation of data modelled as distributions on an underlying space-of-interest, interpolating the path of minimal energy to preserve mass. The CSF-to-PVS Gd uptake model on MRI now accounts for advection and diffusion flow, and image noise, in estimating the movement of Gd through the image to visualise the glymphatic system over several hours (Figure 4).[33] The ability to visualize and quantify dynamic PVS function in humans, perhaps with OMT applied to dynamic intravenous Gd enhanced MRI,[65] could be very powerful when available.

Why do PVS become visible in humans and what does this tell us?

We do not yet know precisely why PVS become visible in people on MRI or at post-mortem, but we know that several factors are associated with increased visibility. These may provide pointers to mechanisms and neurological implications.

Risk factor associations

Firstly, PVS visibility increases with age. In a risk factor adjusted meta-analysis, in 13 studies including 8395 subjects, PVS visibility assessed with visual scores increased with age in the basal ganglia, centrum semiovale and hippocampus.[1] The age-visibility effect was different between the three areas (Chi^2 =7.1, P=0.03), was strongest in the basal ganglia (OR 1.47, 95%CI 1.28-1.69, P<0.00001), less in the centrum semiovale (OR 1.26, 1.07-1.49, P=0.005), and lowest in the hippocampus (OR 1.14, 1.01-1.30, P=0.03).

In terms of other risk factors, PVS visibility increased with hypertension (11 studies, 7872 subjects, mostly treated), significantly in the basal ganglia (OR 1.67, 1.20-2.31, P=0.002) but not in the centrum semiovale (OR 1.42, 0.92-2.20, P=0.12).[1] In five studies (3095 subjects), PVS visibility was not associated with diabetes.[1] There were insufficient data for meta-analysis on smoking, but one study found no association between cigarette smoking and PVS visibility.[63]

An association between PVS enlargement and inflammation is also important to consider since PVS are a site of inflammatory infiltrates on histopathology in SVD. In MS, an inflammatory disease, focal PVS widening has been observed at the edge of active MS plaques at the start of inflammatory exacerbations.[10,66] In one report, these prominent focal PVS disappeared as the MS lesion inflammation resolved.[10] Patients with systemic inflammation (in the form of systemic lupus erythematosus) had increased PVS visibility,[67,68] which was higher in both basal ganglia and centrum semiovale than in healthy age-matched controls, but of similar severity to age-matched patients with minor stroke (despite the former's much lower vascular risk factor profiles).[68] The lupus patients also had more WMH than expected for age, and these features and other measures of brain microstructural damage were associated with minor cognitive impairments[69] and symptoms of fatigue.[70] In a community-based study of ageing of about 700 subjects all aged 72, increased plasma markers of inflammation were associated with increased basal ganglia PVS visibility, which in turn were associated with WMH, but there was no direct association between plasma inflammatory markers and WMH,[71] suggesting that the association seen between inflammation and WMH in other studies may occur secondary to inflammation-related PVS dysfunction, but this requires further testing.

Neurological associations

Visible PVS have been associated with quite a range of neurological conditions, but often in too few studies, too varied populations, or too varied clinical phenotypes, to combine in meta-analysis, making the evidence base sketchy. Seven cross-sectional studies in patients with various types of stroke (n=2855) did not find associations between PVS and stroke subtype, but the subtyping, e.g. of lacunar vs. non-lacunar stroke may have been suboptimal in some studies,[1] since individual studies with optimal subtyping did find that basal ganglia PVS were more prevalent in lacunar than non-lacunar stroke.[55] For recurrent stroke, the picture is also mixed:[5] in 2002 patients with ischaemic stroke or transient ischaemic attack (TIA), recurrent ischaemic stroke but not recurrent haemorrhagic stroke was more frequent in patients with increased PVS visibility; in 229 patients with intracerebral haemorrhage (ICH) and amyloid angiopathy, high centrum semiovale PVS visibility was associated with recurrent ICH; but in 1228 community-based subjects, high PVS visibility was associated with any vascular event and death but not recurrent stroke once adjusted for vascular risk factors.[5]

Data on PVS on MRI and cognitive decline or dementia are conflicting. An early report found that increased basal ganglia and hippocampal PVS were associated with cognitive impairment in patients with insulin dependent diabetes and hyperglycaemic episodes.[44] Three other studies (n=1272) did not find an association of basal ganglia PVS with cognitive impairment, one study found a borderline significant association of centrum semiovale PVS with cognitive impairment, three studies found PVS were increased in patients diagnosed with dementia versus controls, and two found increased PVS were associated with cognitive impairment.[1,3] On future dementia risk, one population-based study (n=1778) found the top grade of visible basal ganglia and centrum semiovale PVS predicted incident dementia, while another population study (n=2612) found that large PVS and basal ganglia PVS predicted a steeper decline in processing speed and increased the risk of incident vascular dementia, but not all dementia or AD.[5 72] In five population-based cohorts of 3575 subjects mean age 63, there was no association between PVS visual scores and current cognitive status,[73] but the subjects may have been too young or healthy, or visual scores too insensitive, to detect subtle associations with cognition.

Other studies of neurological disorders and PVS suggest associations with autistic spectrum disorders, depression, Parkinson's disease, neurolupus, MS, CADASIL,[54] Collagen type IV mutations,[74] and a range of other conditions, but mostly in small studies and frequently without adjustment for vascular risk factors.[1]

Finally the relationship of PVS visibility to mortality is unclear: one study in patients with ischaemic stroke (n=2002) found no association with death, while a population study (n=1228) found PVS were associated with death from vascular causes.[5]

These studies tell us, importantly, that visible PVS associations differ with PVS location, since hypertension, systemic markers of inflammation, lacunar vs. non-lacunar stroke and dementia are more strongly associated with basal ganglia PVS visibility than at other sites. This might reflect several anatomical differences in PVS structure (of which more below) or function, although some caution is required in view of the substantial variation in study methods, populations, and co-variate adjustment.[1]

PVS and other SVD lesions – related, or epiphenomenon?

Human MRI PVS research came to prominence in the mid 2000s,[46] several decades after WMH[75] or lacunes[76,77] had become established, and shortly after microbleeds came to prominence.[78] However, the latter have received far more attention due concern about microbleeds and haemorrhage risk with antithrombotic or thrombolytic treatments.

Early studies suggested that PVS, at least in the basal ganglia, were associated with WMH and lacunes, in patients with stroke or cognitive presentations of SVD,[46,55] and in community-dwelling older people.[63,79] Since large PVS might overlap in size with small lacunes, some sources considered that PVS might be mistaken for lacunes, inflating any apparent association. We examined sizes reported in the literature for PVS and lacunes and found that although the largest reported PVS were 5mm in diameter, most were 3mm maximum, while most lacunes were minimum 3mm diameter in size.[80] Sensitivity of MRI has increased since the 2000s, so that these cut-offs may now need revision. Additionally, we have seen recent small subcortical infarcts of up to 10mm diameter (when acute) resolve to leave a tiny lacune of less than 2mm maximum diameter: therefore PVS size criteria should be seen as a guide but applied with caution, pending further data from longitudinal studies of small subcortical infarct fate. The variation in PVS and overlap with lacune maximum diameter is consistent with pathological findings and may explain the preponderance for lacunes in the basal gangila.[81]

PVS are associated with WMH in many cross-sectional studies – the more visible PVS, the more severe the WMH. While WMH appear to form around PVS (Figure 2), we should consider the implications of a recent systematic review of PVS-SVD lesion associations that questioned whether PVS are actually associated cross-sectionally with WMH.[1] In the 23 studies that adjusted for age and vascular risk factors with meta-analysable data, PVS were associated with lacunes (n=4894, odds ratio (OR) 3.56, 85% CI 1.39-9.14, p=0.008) and microbleeds (n=5015, OR 2.26, 95% CI 1.04-4.90, p=0.04). However, although the direction of effect for WMH was consistent with a positive association, the result was not significant (n=4974, OR 1.54, 95% CI 0.71-3.32, p=0.27). Another eight studies on PVS and WMH (n=3,333) that were otherwise relevant, could not be meta-analysed for various reasons. Individually, seven of these eight non-meta-analysable studies found positive associations between basal ganglia PVS and WMH.[1] Therefore the apparent lack of an association between PVS visibility and WMH in meta-analysis may reflect methods limitations rather than a true lack of association. There are few longitudinal studies of PVS and WMH development. One population-based study found that having large PVS visible on MRI was associated with worsening of WMH over a four-year period, with more severe WMH progression in those with more large PVS, and with worsening lacune formation.[72]

For microbleeds, the data are stronger for an association with basal ganglia PVS (five studies, n=5015, OR 2.26, 95% CI 1.25-4.00, p=0.04) than with centrum semiovale PVS (two studies, OR 1.15, 0.62-2.12, p=0.66) with insufficient data to test lobar versus deep microbleeds. Having more large PVS was associated with worsening of microbleeds over a four year period.[72] Focally prominent PVS can occur deep to areas of cortex affected by superficial siderosis[82] (another manifestation of CAA), supporting the idea that PVS dilation may be a sign of blocked drainage pathways due to amyloid-β deposition,[15] as demonstrated at post-mortem (Figure 2c).[56] However, PVS become visible in the white matter up to the inner cortical margin in many other non-CAA related situations (Figure 2a), indicating that the appearance cannot be CAA or β-amyloid specific. Thus PVS dilation in the immediate subcortical white matter may reflect impaired fluid drainage, particulate or protein deposition, excess fluid leakage from the vasculature,[83,84] and stagnation for any reason. This has led to the concept of ‘protein elimination failure angiopathies’ (PEFA).[8,27,58] Interestingly, despite the massive interest in β-amyloid protein in AD, the amyloid-β protein was originally isolated at post-mortem from meningeal vessels

affected by severe CAA,[58] providing another link between vascular and neurodegenerative disease.[85]

Related variables should form a 'latent trait', i.e. a derived variable that reflects the shared variance of the actual variables from which the latent variable is constructed. We tested if the four commonly observed MRI features of SVD (WMH, lacunes, microbleeds and PVS) could be considered together as a 'total SVD burden' latent variable, using data from about 700 older community-dwelling subjects.[79] The four MRI markers did indeed form a unitary 'total SVD burden', similar to a summed SVD burden score, both of which were associated with lower general cognitive ability in older age. All the individual SVD variables contributed significantly to the latent SVD variable, thus confirming that the individual SVD features contribute to the SVD total burden latent variable. The magnitude of effect was such that for each standard deviation (SD) increase in the total SVD latent variable, the PVS score increased by one point (out of a total of 4, details below), the WMH by 1.6, lacunes by 1.2 and microbleeds by 1.7. Therefore, in addition to examples above showing WMH apparently forming around PVS in the centrum semiovale (Figure 2) and PVS-microbleed associations, it is probably reasonable to work on the principle that PVS are associated with the other visible SVD lesions, including WMH. However, evidence for a causal role of PVS in SVD lesion formation is very limited and it is important to consider that MRI-visible PVS may also be an epiphenomenon, or possibly represent an early stage in development of other SVD lesions.

PVS anatomy and physiology

Unfortunately, the anatomy of PVS is complicated and controversial (Box 2). In mammals, the perforating arterioles are surrounded by pia mater which envelopes the vessels as they pierce the brain surface and run deep into the brain (Figure 4 and 5).[8,30] As the arteriole tapers, the pial membrane is replaced at the capillary level by a basal lamina and then surrounded by a 'cuff' of astrocyte end-feet (Figure 6).[30,40] The space between the arteriolar smooth muscle basement membrane and the enveloping pia mater is generally referred to as the 'Virchow-Robin space' and corresponds with the PVS that are visible on MRI.

The spaces around arterioles entering the basal ganglia are invested in two layers of leptomeninges with the PVS lying between these layers (Figure 3C) and communicates with the basal subarachnoid space in rodents[26] and humans.[4] Arterioles entering the brain via the cortex, and all venules, have one leptomeningeal

layer.[56] There is debate about whether the convexity cortex periarteriolar spaces communicate with the subarachnoid space, or only with the subpial space (see earlier and below).[26,81,86,87] Early rodent histological studies suggested that the convexity cortex periarteriolar space only reached the subpial space.[26,81,86,87] These studies used post-mortem tissue, and a recent study in live mice showed that perfusion fixation resulted in abnormal retrograde flow within and collapse of the PVS, with tracer distributed into the vessel wall (Figure 6).[13,88] Cortical PVS must communicate with the subarachnoid CSF at some point, since tracer administration into the cisterna magna CSF in live rodents is rapidly taken up in convexity cortex PVS along the MCA, as well as in basal ganglia (Figure 4).[89]

Furthermore, in humans after injection of the MRI contrast agent Gd into lumbar CSF, contrast was seen in convexity cortex of all brain regions,[90] starting by 6-9 hours after injection and reached throughout white matter by 24 hours (Figure 5),[91] somewhat similar to the distribution pattern in rodents. In patients being investigated for normal pressure hydrocephalus versus 'controls' (patients being investigated for suspected CSF leak), there was reduced Gd uptake into basal ganglia PVS,[92] more Gd reflux into the lateral ventricles and periventricular white matter,[91] and delayed clearance of Gd from CSF, the entorhinal cortex and adjacent white matter.[93] Strangely, little Gd was seen in basal ganglia PVS despite these being a prominent locus of PVS visibility on MRI in humans,[1] of tracer uptake in rodents (Figures 4 and 5), and of open connection from the basal cisterns into the basal ganglia PVS (Figure 2A inset).[48] Alternatively, the differential ventral or dorsal PVS uptake might also reflect that rodents are typically studied in the prone (ventral side down) position with 2-PI, and supine (dorsal side down) in MRI,[48] whereas humans are typically scanned supine (dorsal side down). Gd is heavier than CSF so will preferentially gravitate to lower locations, and body position affects CSF tracer/contrast influx in rats significantly.[94]

CSF and ISF connections

Humans have about 140 ml of CSF, which is produced continuously in the choroid plexus, passes through the ventricles to the subarachnoid space. CSF can drain into the sagittal and transverse venous sinuses via arachnoid granulations which are clearly visible structures on human MRI, although the extent of their involvement in CSF/ISF drainage has recently been questioned.[92] In humans, the dural venous sinuses also have many arachnoid villi that are not visible on MRI. The number and location of arachnoid granulations are said to be different in the rodent, and mainly

present only in ventral venous sinuses, and the extent of their involvement in CSF/ISF clearance is unclear. Although still debated, other sources likely contribute to net CSF production, including the ISF generated from the cerebral microcirculation via the BBB[25] and as a byproduct of brain metabolic activity. Some ISF may return to the circulation by exchange across the BBB, resorption into capillary-venular blood and thence to drain out with the blood.

Many review papers include diagrams suggesting that most ISF is thought to leave the brain via the perivenular PVS,[29,89,94,95] but it is unclear if the perivenular PVS connect to subarachnoid CSF or remain subpial,[8,26,27] or if they are the ISF exit conduits.[17,27,29,40,53,96] Most original papers focused on periarteriolar spaces, resulting in less data on perivenular spaces. Thrane *et al* showed lipophilic and hydrophilic fluorescent tracers appearing in the perivenular space at 60-90 mins after cisternal injection in mice.[97] However there are few other published images (perhaps only one convincing image[89]) of contrast reaching perivenular spaces *by any method*. This near total absence of evidence of fluid reaching perivenular spaces might reflect the short duration of experiments focusing on periarteriolar space function, dilution of tracers, differences between physiological and pathological states, or fixation artefacts.

Other studies differentiated between ISF drainage and CSF uptake, showing that cisternal CSF tracers entered the brain along pial-glial membranes, leaving the brain along capillary basement membranes and arteriolar smooth muscle cells' basement membranes against the direction of blood flow, eliminating ISF and solutes from the brain,[98,99] without connecting to perivenous drainage spaces.[99] In a pathological situation such as β-amyloid deposition, in patients with CAA or Alzheimer's disease studied post mortem, in rodents that overexpress β-amyloid, or following β-amyloid injection into the hippocampus,[98] the β-amyloid is deposited in pericapillary and periarteriolar but not perivenular membranes[17] (see[17] for an in depth review of β-amyloid and its clearance from the brain). Smooth muscle cells and pericytes are highly phagocytic and the periarteriolar/pericapillary localization of vascular amyloidosis may reflect that contractile cells are sparse around venules. Tracers injected into the rodent brain parenchyma appear to drain mainly along arterioles, although striatal injection could be considered pathological[100] similar to seeing fresh haemorrhage tracking in basal ganglia PVS in humans (JMW, personal observation). Increased PVS visibility is a marker of BBB leak in patients,[83] where signal due to Gd was visible in the basal ganglia PVS at 30 mins after *intravenous* injection, and can

only have reached there by crossing the BBB *somewhere* (Figure 5d). How the Gd entered the PVS is less clear, since it was not possible to track its pathway in real time. It could have crossed into CSF at the choroid plexus, travelled out of the ventricles and started to be flushed back up the PVS, although that seems unlikely in only 30 mins.[91] It could have collected in the PVS from ISF, although tracers injected into CSF are seen moving up the basal PVS in rodent and human studies in vivo. Most likely, the Gd leaked from the arterioles and/or capillaries into the PVS as shown recently in pericyte-deficient mice.[101] These mice develop a substantial early BBB breakdown leading to a large increase in the number of enlarged PVS in white matter, suggesting that pericyte degeneration causing BBB breakdown results in increased fluid in (and visibility of) the PVS.[101] Pericyte loss and BBB breakdown are found in neurological disorders associated with visible PVS in humans[1] and in rodent models, including AD[84,102] and CADASIL,[103] providing a mechanistic explanation for the association between BBB breakdown and PVS visibility in patients,[65] but not helping to solve the conundra of ISF drainage routes (Box 2).

Meningeal and nasal lymphatics

Meningeal lymphatics drain alongside the major dural venous sinuses, via cranial perineuronal channels[26,98] to cervical lymph nodes,[8,104] and are thought to be major ultimate drainage routes for interstitial fluid. Dural lymphatics were in fact described in humans, rodents and sheep many years ago (summarised in[26]) although their dynamic function was documented more recently.[104,105] Probable small dural lymphatics were recently demonstrated with difficulty in humans in vivo on delayed MRI after intravenous Gd injection with specific MR sequences.[28,106] Dural lymphatics along the major venous sinuses were demonstrated recently in a transparent mouse preparation in which dye injected into the CSF in vivo was taken up into dural lymphatics.[107]

Rodents, rabbits, pigs, sheep, monkeys and humans have nasal lymphatics (Figure 4) which drain from the frontal subarachnoid space, through the cribriform plate via the nasal mucosa and thence to cervical lymph nodes,[8,26,108] although most data are post-mortem. Tracer injected in vivo into the cisterna magna in rodents flows forward and exits the cranial cavity via nasal lymphatics to the cervical nodes (Figure 4).[26] In patients undergoing lumbar CSF injection of Gd to investigate CSF circulation disorders repeated brain MRI for 48 hours showed altered signal in cervical lymph nodes attributed to Gd, but the precise route whereby the Gd reached the cervical lymph nodes is unknown.[90] PET imaging in patients with AD and healthy volunteers

showed that *intravenously* injected tau radiotracer concentrated in the nasal turbinates.[109] Further research is required to determine if *intrathecally* administered radiotracers can determine where tau, β-amyloid and other relevant molecules exit the cranial cavity, and which routes are the most important in humans.

The relative proportion of ISF draining in humans via nasal lymphatics, peri-arterial or peridural lymphatics to blood or to cervical lymph nodes, versus via arachnoid granulations and villi into the major venous sinuses, is unclear. In a transgenic mouse without dural lymphatics, the macromolecule clearance from the brain was attenuated and fluid drainage to cervical nodes was diminished, but there was little effect on brain ISF drainage or intracranial pressure, suggesting that most fluid flow exits the brain by alternative routes.[105] Inter-species differences may account for some gaps in understanding, most studies of CSF and ISF having been in rodents, rabbits or sheep.[8]

What drives fluid movement through the PVS and brain?

Movement of fluid through the brain depends on several factors including vascular pulsation,[13,14,87] respiratory movement,[110] and probably the sleep-wake cycle.[12] A large volume of blood, approximately a litre (a fifth of the cardiac output), passes through the human brain every minute at rest, more during increased brain activity. The ISF has to be exchanged and metabolic waste has to be removed. The turnover and volumes are such that an active removal system seems necessary to maintain good 'brain hygiene', leading to the recent concept of the 'glymphatic' system,[111] a system of fluid flushing and drainage from the brain that uses pathways running along around arterioles, capillaries and venules. Unlike systemic vascular beds, where arterial pressure and flow pulsations dissipate to become essentially continuous at the arteriolar and venous level, within the rigid skull the limited tissue compliance promotes propagation of arterial pressure pulsation throughout the brain leading to measurable pulsatile flow in the microvasculature and venous outflow.[52] This preservation of pulsatility along the entire vascular bed may help to 'push' fluid and waste through the brain, and may explain why periarteriolar rather than perivenular spaces dilate in disease. Periarteriolar space shape appears to play a role in efficient fluid shift, the optimum shape being oblate (a sort of double-pointed teardrop) with the arteriole lying eccentrically.[13,88] Speculatively, minor changes in CSF or tissue pressure, or in vascular function, that altered the PVS shape could accelerate dysfunctional PVS flow and secondary drainage problems.

Aquaporin 4 (AQP4) and astrocytes: Movement of CSF into the ISF space and active flushing are thought to be facilitated by AQP4 water channels on astrocyte endfeet which wrap around capillaries,[89] although AQP4, and its role in promoting PVS/ISF clearance, are debated.[21] Some questioned the importance of AQP4, based on the observation that CSF tracer uptake in mouse brain and interstitial flow rate of fluorescent dextrans were consistent with diffusion, unaffected by cardiac arrest or by AQP4 knockout,[21] while others also supported diffusion-based CSF para-arteriolar flow.[112-115] However, five groups used different AQP4 knockout mouse strains and showed that AQP4 was necessary for the rapid passage of fluid into and through the brain.[116] Different results may reflect differences in technique and/or anaesthetic agent .[53,100] Data on human AQP4, vascular and PVS function are very limited.[117] In brain biopsies from patients with normal pressure hydrocephalus (mean age 70.8+/-8,8), AQP4 on astrocyte end feet was reduced compared with younger controls undergoing brain surgery for other reasons (mean age 44.0+/-16.5)[118] but the huge age disparity renders these results unreliable since all known aspects of cerebral haemodynamics change with aging (e.g. resting blood flow falls,[119] PVS function diminishes[47,120]). Other studies in post-mortem (PM) samples from patients with stroke or AD with better age matching found that aberrant location of AQP4 on astrocyte endfeet, astrocyte damage and BBB disruption were associated with impaired cognition in life,[117,121,122] but these may be co-associations rather than causations.

Vascular pulsation: In rodents, various techniques demonstrate that movement of fluid along the PVS is assisted by pulsation in the arteriole at the centre of the PVS (Figure 6).[13,14,87] Microparticles injected into the cisterna magna can be seen tracking up PVS using 2-PI via cranial windows (Figure 6). At normal blood pressures, the arteriolar pulsation and particle movement is smooth, continuously forward, without sticking.[13] During acute blood pressure elevation, the arteriole becomes stiffer, the pulsations in distal vessels increase in amplitude resulting in a 'jerky' movement, intermittent reversed flow, leading to a reduction in net flow in the PVS and failure to clear debris (see video of particle tracking at normal and elevated BP at https://static-content.springer.com/esm/art%3A10.1038%2Fs41467-018-07318-3/MediaObjects/41467_2018_7318_MOESM6_ESM.mov).[13]

Evidence of a similar effect of arteriolar pulsatility on PVS function in humans comes from phase contrast MRI which showed that as intracranial vascular pulsatility increased (particularly venous sinus pulsatility), PVS visibility increased.[52;123]

Interestingly, this approach also demonstrated that the increased intracranial vascular pulsatility was associated with reduced CSF fluctuation at the foramen magnum and in turn with increased basal ganglia PVS. suggesting that a decline in CSF stroke volume resulting in less effective PVS flushing and fluid stagnation.[123] The increased intracranial vascular pulsatility on phase contrast MRI was also associated with faster transmission of the vascular pulse wave through the brain and worse WMH.[52] If the association between foramen magnum CSF stroke volume and CVR, PI, and PVS are confirmed in other studies,[52;123] then phase contrast MRI could be a powerful non-invasive tool to assess intracranial haemodynamics and pathology in humans.

Respiratory motion results in cyclical craniad and caudad movement of CSF at the foramen magnum in humans: inspiration increases CSF flow into the ventricular system and the opposite occurs in expiration.[110] It is possible to see CSF motion around the foramen magnum and in epidural veins in the spine:[124] in forced inspiration, as the venous blood is drawn down into the thorax, the CSF moves up in to the cranial cavity towards the brain offering a way that respiratory motion may contribute to the PVS flushing process, perhaps providing another reason for sighing (beyond re-inflating lung alveoli) although this remains to be demonstrated in humans.[124]

PVS function and sleep

Studies in rodents show increased uptake of CSF into PVS and flushing of the ISF during sleep.[12] However, there are very limited data on whether and to what extent human PVS function is altered in healthy sleep and disrupted in disordered sleep. It could be argued that the human brain is larger than the rodent brain and thus requires constant replenishment of ISF, but there is no information on this.

In patients with undergoing investigation for hydrocephalus in whom Gd contrast agent was injected into the lumbar CSF, uptake of Gd into PVS was increased during the night compared with daytime, despite patients having their sleep disrupted through repeated scanning.[92,93] Furthermore, there is indirect evidence of PVS dysfunction during disrupted human sleep. Increased PVS visibility was associated with obstructive sleep apnea (n=170)[125] and was associated with reduced sleep efficiency on polysomnography in a small study of patients with cerebrovascular disease (n=26).[11] In a population-based study, increased PVS in the basal ganglia were associated with impaired sleep efficiency on polysomnography.[126] In healthy

persons, one night's sleep deprivation resulted in increased amounts of β-amyloid in the thalamus and medial temporal structures on β-amyloid PET imaging.[127] In a controlled experiment, disruption of slow wave sleep led to higher CSF β-amyloid the following day; worse sleep efficiency in the week prior to lumbar puncture was associated with increased CSF tau, which was interpreted as the sleep disruption increased neuronal activity and generated more proteins.[128] An alternative explanation is that disrupted sleep reduced natural PVS flushing resulting in less β-amyloid and tau clearance.

Both ISF production and clearance should be considered. Recent work in mice showed that increases in hippocampal ISF and CSF tau were closely related to neuronal activity but not to paravascular clearance, and in patients that CSF tau and β-amyloid levels were increased by sleep deprivation and related to increased production.[129] The picture is complex. In addition to increased clearance of β-amyloid during sleep in mice from accelerated ISF-to-CSF glymphatic-mediated bulk flow,[12,130] the BBB may facilitate clearance.[17] Under awake conditions, 85% of β-amyloid is removed via transport across the BBB and 15% via passive ISF bulk flow,[131,132] while during sleep, β-amyloid clearance is increased by about 2.2 fold of which 60% occurs via BBB transport and 40% by ISF flow.[12,17] Thus changes in CSF tau and β-amyloid may reflect multiple factors, including production of proteins and other substances from cell activity, as well as changes in clearance with sleep-wake cycles.

Translation to the clinic

What does it mean if a patient is noted to have many PVS on their MRI? As described in the earlier sections on risk factors, diseases and brain lesions, increasing numbers of visible PVS on MRI are an indicator of impaired brain health, albeit non-specific. A few PVS are commonly seen in the basal ganglia or centrum semiovale at most ages. However, seeing many PVS, particularly at younger to middle age, may be clinically important, but unfortunately is a non-specific finding at present. The interpretation and action will depend on several factors. How old is the patient? What was the indication for the scan? Is there a history of hypertension, is their BP normal, are there other vascular risk factors, or other SVD features such as WMH or microbleeds? Is there any cognitive impairment? Is there any systemic inflammation? The forgoing are all factors and disorders associated with PVS which might provoke more detailed assessment of modifiable risk factors and appropriate

interventions. These might include blood pressure reduction if the patient is found to have hypertension, or efforts to improve glycaemic control if the patient is diabetic, for example.

In order to make better use of information from PVS, much more information is needed. Better understanding of how many PVS can be considered 'normal' at different ages is required. Simpler methods to quantify PVS are needed to assist clinical services in identifying when PVS are exceeding normal limits. This might be simpler visual rating scales or better computational methods. Much more data are required from long term studies to determine the current clinical relevance and risk of future diseases, notably stroke and dementia, of finding excessive numbers of visible PVS, particularly in patients of younger ages. At least some improved understanding of the implications of PVS for future health could be obtained by more analysis of existing datasets of young and middle-aged population cohorts that are now in long term follow-up, or (where available) through 'big data' analysis of routine clinical MRI plus central health data linkage to ascertain future incident diseases. New studies should also include a T2 volume sequence to optimise PVS detection and include long-term follow-up for cognitive and vascular disease outcomes.

Conclusions/perspective

Our understanding of PVS is better than it was, but still patchy. Fluid and metabolite management through the brain is a difficult topic to research, reflecting the demands of a system designed to supply energy to a highly complex electrical organ while flushing out waste, all without unbalancing the delicate milieu necessary for neurons to function, and making it very difficult to perform experiments that do not inadvertently upset this delicate balance. We know that increased visibility of PVS in humans on MRI is a marker of age, several vascular risk factors, inflammation, BBB leak, occurs in conjunction with other SVD features, associated with cognitive decline and, by extrapolation from post-mortem and rodent studies, is a marker of impaired brain ISF flushing.

However, there are several unexplained observations (Box 2). The PVS seen on MRI in humans are most likely periarteriolar, CSF uptake is most pronounced in periarteriolar spaces in rodents, but tracers injected i.v. or intrastriatal are also seen around arterioles in humans and rodents, yet ISF should drain via perivenular spaces. Few perivenular spaces have been described or imaged. Perhaps

perivenular spaces are more difficult to visualise since rodent experiments would have to last much longer, or the perivenular tracer is diluted by continual wash in of fresh CSF/ISF? Tracers indirectly image fluid movement, are affected by other forces in tissues, molecular interactions and weight, and effects of extracellular space geometry that reduce their effectiveness at tracing the fluid movement. Ethical issues also preclude injecting Gd into CSF in humans, the few studies so far were in patients undergoing investigation for abnormal CSF dynamics.[91,133] It is unclear where the perivenular fluid drains to, but rodent studies suggest via the subpial (and/or subarachnoid) spaces to dural and nasal lymphatics (Figure 4) and cervical lymph nodes.[8,134] Lack of studies and major technical challenges mean that there is limited evidence for dural lymphatics in humans, for connection to cervical lymph nodes, or to determine the relative amounts of intracranial fluid draining by each route, in physiological and pathological conditions. The route by which excess β-amyloid, if generated in the brain, deposits in periarteriolar spaces and arteriole walls is unclear. The AQP4 water channels on astrocyte end feet are considered very important in promoting ISF flow through the brain, yet little is known of AQP4 function or abnormalities in the human brain in cognitive or vascular disease states.[121,135]

There are major implications for future research. Differences between rodent and human brains mean that the more that studies can be done in humans, the more relevant the results are likely to be. On the other hand, pre-clinical studies in animals allow invasive manipulations that are not possible in humans, that are likely to shed light on mechanistic aspects of PVS, and their relationship with disease, as long as the physiological versus pathological implications of the methodology are considered. New imaging approaches to visualize fluid flow directly, avoiding the limitations of current tracers, would be very helpful, if reliable. In vivo MRI methods now allow the measurement of PVS shape, size, number, location, plus intracranial vascular and CSF pulsatility, flow, CVR, BBB function, etc. Large-scale longitudinal studies are now required to understand how PVS dysfunction starts, how this affects ISF balance, how it leads to brain damage, and whether and how this can be reversed. Studies are needed to determine the relative roles of dural lymphatics versus other drainage pathways, and the role that cervical lymph nodes have in maintaining the brain's immune competence. And clarification is needed of the clinical implications of increased PVS visibility on brain MRI performed for clinical investigation.

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Links to web sites:

The Leducq PVS website: www.small-vessel-disease.org

The HARNESS website: https://harness-neuroimaging.org/

Box 1: Key points

1. Visible PVS on MRI increase with age, vascular risk factors particularly hypertension, and other small vessel disease features, indicating that visible PVS are clinically relevant, not merely epiphenomena;
2. PVS dilation on MRI is a marker of PVS dysfunction and, by implication, impairment of normal brain fluid and waste clearance and microvascular dysfunction;
3. PVS can be quantified on MRI in humans using visual scores of PVS number in standard brain regions, and now also with computational measures of PVS count, volume, length, width, sphericity, and orientation;
4. Experimental models show that PVS are important conduits for uptake of CSF to flush ISF and clear metabolic waste, which may increase during sleep, but this remains to be demonstrated conclusively in humans;
5. The importance of the different routes of PVS drainage, CSF resorption and fluid proportions taking each route, including via dural and perineural lymphatics and cervical lymph nodes, have undergone very limited study and remain to be determined in humans.

Box 2: Big Questions

1. Do perivenular spaces drain ISF? If so, where to?
2. Once out of the brain, what proportions of CSF or ISF reach sub-pial or sub-arachnoid spaces, and drain via dural lymphatics or arachnoid granulations and villi?
3. If ISF drains via perivenular spaces, why does β-amyloid and other debris sequestrate in periarteriolar spaces?
4. In humans, are most PVS visible on MRI periarteriolar, perivenular, or both, and is it possible to distinguish these?
5. In humans, to what extent do vascular pulsation and/or respiratory effort facilitate fluid movement through PVS and the brain extracellular space?
6. In humans, does AQP4 facilitate uptake of CSF into PVS and flushing of the ISF?
7. In humans, does PVS function differ during sleep versus waking?

Review Criteria

We used recent systematic reviews where available and updated their contents. We searched the literature from the mid 1800s to the present for papers on ‘perivascular spaces’, ‘glymphatics’, ‘Virchow-Robin spaces’, ‘small vessel disease’, ‘CSF’, ‘cerebral blood flow’, ‘white matter hyperintensities’, ‘lacunes’, ‘microbleeds’, ‘siderosis’, ‘stroke’, ‘dementia’, ‘cognition’, and methodologies like ‘magnetic resonance imaging’, ‘2-photon imaging’, ‘electron microscopy’, and ‘immunohistochemistry’. We checked reference lists in review and original papers. Our approach was not systematic given the breadth of the field, but we aimed to capture key papers in the field. We discussed and debated at length the historical and more recent findings in our Leducq research network.

Figures

Figure 1. Perivascular spaces as visualised on magnetic resonance imaging in people.

A, top illustrates typical PVS on T2-weighted MRI in the temporo-occipital region extending as thin white lines from the lateral ventricle towards the cortex; bottom, schematics representing the appearance of PVS on T2-,T1-weighted and FLAIR images, longitudinally when in the plane of the image and circular when perpendicular to the imaging plane (adapted from STandards for ReportIng Vascular changes on nEuroimaging, STRIVE[7]).

B, T2-weighted MRI illustrating different severities of PVS in two standard regions, top, basal ganglia, and bottom, centrum semiovale, Visual rating scores (1 to 4) are indicated along the bottom.[43]

C, Axial view of computational identification of PVS, (i) moderate and (ii) severe. (Adapted from Fig 4 in[31]).

D, Comparison of computed total PVS volume and (i) WMH visual score and (ii) WMH volume in 500 community-dwelling subjects aged 71-73.

Figure 2. Perivascular spaces in people on high and conventional field strength magnetic resonance imaging and at post-mortem.

A, A coronal high field (7T) MRI T2 (large image), and magnified image of perforating arteriole and PVS in basal ganglia: T2 (small B), T1 (small C) and MRA (small D) of basal ganglia PVS. Note inferior ends of PVS widen to join the basal CSF (arrows). Reproduced from Fig 3, Bouvy et al Invest Radiol 2014;49:307-313,[4] (permission requested from the publisher Wolters Kluwer via Rightslink).

B, two T2-weighted images of frontal lobe of different subjects at 1.5T show PVS approaching the cortex and appearing more dilated towards the inner cortex edge, but not visible passing through the cortex.

C, PM Hematoxylin and eosin-stained superior frontal gyrus and white matter sections from (i) a cognitively normal 74-yr old subject with ApoE ε3/ε3 genotype, who died of non-brain disorder showing no noticeable PVS enlargement, and (ii) an 80-y-old AD patient with ApoE ε4/ε4 genotype showing numerous arterioles with enlarged PVS throughout the entire visible white matter that do not propagate through the cortex; the paler blotches in the white matter represent areas of myelin rarefaction. Magnification: about 2.5×. Reproduced from Roher et al Molec Med 2003;9:112-122, fig 3.[56]

D, Axial 1.5T MRI of 75 yr old subject presenting with minor ischaemic stroke. T2-weighted image (i) shows numerous PVS (circled) running in white matter perpendicular to cortex, while FLAIR (ii) image shows WMH starting to form around the PVS including some larger WMH (lower circle), similar to the appearance in C ii of linear and more blotchy white matter rarefaction.

Figure 3. Perivascular spaces, relationship to arterioles, venules, and morphology by brain location, in people.

A), left, high field, 7T MRI shows PVS (small A) are spatially aligned with arterioles not venules. Reproduced from figure 5, Bouvy et al Invest Radiol 2014;49:307-313,[4] permission requested from the publisher Wolters Kluwer via Rightslink.

B) 1.5T MRI top adjacent T2-weighted images, bottom T2* image. PVS (white arrows) do not align with venules (black arrows and arrowhead) but run parallel to the PVS.

C) Diagram of meninges surrounding basal (left) and cortical (right) perforating arterioles. Basal arterioles have two meningeal membranes whereas cortical arterioles and all venules have only one meningeal coating, thus are thought to communicate with the subpial space,[81] despite which tracer injected into subarachnoid CSF appears to reach cortical PVS (see Figure 5). Reproduced from fig 6 in Pollock et al J Anat 1997;191:337-346.[81]

Figure 4, Glymphatic transport in whole rat brain visualized by optimal mass transport (OMT) and GlympVis.[33]

Three lateral volume rendered images of a normal rat brain obtained with MRI: olfactory bulbs are to the left and brainstem to the right of each image. A Gd contrast was injected into the cisterna magna CSF of the anaesthetized rat and the rat scanned for 1.5 hours in the supine position.

A) Shows 'static' visualization of glymphatic transport as a color-coded map representing the sum of all images over 1.5 hrs after Gd injection. The color-coded map, overlaid on the grey anatomical whole rat brain mask, shows the spatial distribution of Gd in the CSF and demonstrates that CSF has penetrated into the cerebellum, midbrain, ventral surface of the cerebral hemispheres, the PVS around the MCA, and into the olfactory bulb.

B) Same data as in 'A', processed using GlymphVis which includes advection and diffusion terms.[33] The color-coded map now represents glymphatic CSF flow trajectories (streamlines) which indicate CSF flow patterns at a fixed point over 1.5 hours and are visible within the brain parenchyma (the anatomical brain mask is rendered partly transparent).

C) Same data as in 'B', however the surface rendering of the rat's brain now enhances visualization of CSF streamlines on the surface of the brain and out of the brain (drainage). CSF streamlines can be appreciated along the PVS of the MCA. Furthermore, CSF streamlines are visible exiting along the olfactory nerves and around cranial nerves (VIII and V) at the level of the brain stem.

Scale bar = 2mm. Figure courtesy H Benveniste, A Tannenbaum.

Figure 5. Uptake of CSF into PVS in rodents and humans.

A. Mouse (Ai-ii) and rat (Aiii-iv) coronal brain slices taken just anterior (i, iii) and posterior (ii, iv) to bregma at 30 minutes after intracisternal infusion of Texas Red-conjugated dextran (TR-d3, MW 3kD) show similar tracer uptake distribution into cortex between species; uptake is less in rat possibly due to its larger size. Imaging is performed with the rodent ventral side down. Reproduced from fig 2 A,b,d,e in Yang et al. J Trans Med 2013;11:107.[136]

B. Series of coronal (top) and axial (bottom) T1-weighted MRI from one patient undergoing investigation for neurological disorder by injecting gadolinium into the lumbar spinal CSF: left to right, baseline, 1-2, 6-9 and 24 hours after injection. Patient remains lying supine throughout and is imaged supine. Note the increased uptake of gadolinium into the cortex with relative sparing of the basal ganglia. Reproduced from Figure 1, in Ringstad et al JCI Insight 2018;3(13):e121537.[91]

C. High magnification micrographs of coronal sections at the level of the bregma of the hypothalamus showing the fluorescence intensity of tracer TRd3 injected intracisternally and imaged at 30 minutes after injection. Tracer is within the PVS (star) and adjacent brain parenchyma (dotted line) of WT mice (C i) but there is much less uptake in Aqp4 KO mice (C ii). Compare morphology of PVS with the image of human basal PVS in Figure 2. Reproduced from Figure 2e in Mestre et al. eLife 2018;7:e40070 doi: 10.7554/eLife.40070.[116]

D. Axial FLAIR MRI of patient with prior lacunar ischaemic stroke (D i, blue arrow) and white matter hyperintensities, imaged pre (D i) and 25 minutes post (D ii) intravenous gadolinium. Note the increased signal in the basal ganglia PVS (D ii, white arrows) and in the cortical sulci (D ii, yellow arrows) after iv injection; the contrast can only have reached the PVS by crossing the blood-brain barrier. It seems unlikely that there would have been sufficient time for the contrast to reach the basal PVS by first passing into the CSF outside the brain and then being washed back into the PVS.

Figure 6. In vivo dynamics of PVS function are significantly altered after perfusion-fixation. Inset shows position of cranial window access.

a) CSF flow imaged through a cranial window using 2-PI in live mice after fluorescent microspheres were infused into the cisterna magna. Superimposed trajectories of tracked microspheres show that particles are transported primarily in large PVS.

b) Fluorescent dextran in the CSF appears primarily around pial arteries (blood vessels labelled with iv dextran).

c) After fixation, the vessel collapses and the tracer redistributes around the arterial wall. The green tracer appears yellow due to co-localization with the i.v. lectin used to label the luminal wall of the vasculature.

d) The size of the PVS relative to that of the artery, was quantified as the ratio of the area of the PVS over the area of the adjacent artery for in vivo measurements utilizing tracked particles and dextran dye and after fixation. The PVS area is roughly 1.4 ± 0.1 times larger than the arterial area in live mice, and fixation reduces this ratio to 0.14 ± 0.04. One-way analysis of variance (ANOVA) post hoc Tukey's test, $^{***}P < 0.0002$, ns not significant, mean ± SEM, $n = 6–7$/group. Scale: 40 µm. Images from Fig 1 and 2, Mestre et al, Nature Comms 2018.[13]