nature reviews neurology

https://doi.org/10.1038/s41582-023-00884-1

Review article

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Molecular biomarkers for vascular cognitive impairment and dementia

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Abstract

As disease-specific interventions for dementia are being developed, the ability to identify the underlying pathology and dementia subtypes is increasingly important. Vascular cognitive impairment and dementia (VCID) is the second most common cause of dementia after Alzheimer disease, but progress in identifying molecular biomarkers for accurate diagnosis of VCID has been relatively limited. In this Review, we examine the roles of large and small vessel disease in VCID, considering the underlying pathophysiological processes that lead to vascular brain injury, including atherosclerosis, arteriolosclerosis, ischaemic injury, haemorrhage, hypoperfusion, endothelial dysfunction, blood–brain barrier breakdown, inflammation, oxidative stress, hypoxia, and neuronal and glial degeneration. We consider the key molecules in these processes, including proteins and peptides, metabolites, lipids and circulating RNA, and consider their potential as molecular biomarkers alone and in combination. We also discuss the challenges in translating the promise of these biomarkers into clinical application.

Sections

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Key points

- VCID has multiple underlying pathologies that can contribute to cognitive impairment.
- Identification of molecular biomarkers that can differentiate VCID from healthy ageing and Alzheimer disease remains challenging.
- Multiple molecular biomarkers have been associated with VCID, but none has yet been translated into clinical application.
- The heterogeneity and complexity of VCID means that use of multiple biomarkers in combination tends to be necessary.
- Promising biomarkers related to various pathophysiological pathways could be combined into panels to optimize sensitivity and specificity; machine learning could be useful for constructing these panels.

Introduction

Vascular cognitive impairment and dementia (VCID; Box 1) is the second most common cause of dementia after Alzheimer disease (AD), accounting for at least 20% of all dementia diagnoses[1]. Furthermore, cerebral vascular pathology often coexists with AD pathology[2] and is a key contributor to the clinical cognitive profile of AD[3].

Advances in neuroimaging and molecular biomarkers are improving diagnostic precision for dementia, particularly AD[4], illustrated by the development of the National Institute on Aging and Alzheimer's Association research framework for diagnosis on the basis of biomarkers for amyloid-β (Aβ), tau and neurodegeneration, known as the AT(N) framework[5]. For VCID, however, diagnosis currently relies largely on clinical information and neuroimaging, which provide lower sensitivities and specificities than molecular biomarkers. Identification of clinically useful molecular biomarkers of VCID could improve diagnosis and management of the condition.

In this Review, we examine current knowledge of molecular biomarkers of VCID and consider their potential in the clinical management of the condition. We first provide an overview of the pathophysiological pathways that are implicated in cerebrovascular diseases associated with VCID, including small vessel disease (SVD), large vessel disease (LVD), cerebral cardioembolism and intracranial haemorrhage. We focus on SVD, as this condition is most commonly associated with VCID[6]. We then set out the fluid and cellular biomarkers of these pathophysiological pathways and consider whether these biomarkers, either alone or in combination, have clinical potential in the diagnosis, prognosis and monitoring of VCID and how they can be translated into clinical application.

Pathophysiological processes of VCID

The fundamental characteristic of VCID is that cognitive deficits are attributable to brain injury that results from cerebrovascular disease. This brain injury encompasses macro-infarction, micro-infarction, ischaemic lesions and intracranial haemorrhage, which are associated with LVD, SVD and heart disease of various types (Table 1). Several pathophysiological processes that underlie cerebrovascular disease have been identified on the basis of brain imaging and post-mortem studies in humans and/or evidence from animal models. These processes are described in detail elsewhere[3,6], but the following sections briefly describe the major pathophysiological mechanisms to provide context for discussion of molecular biomarkers.

Small vessel disease

SVD is a collective term for pathological changes in small vessels (small arteries, arterioles, venules and capillaries), which manifest as lacunes, white matter lesions, cerebral microbleeds and enlarged perivascular spaces[7]. Underlying these diverse pathological changes are acute ischaemia and/or chronic hypoperfusion, which lead to endothelial dysfunction, breakdown of the blood–brain barrier (BBB), oxidative stress, inflammation, clotting pathway dysfunction and degeneration of neurons and glial cells[6] (Fig. 1).

Endothelial dysfunction. Studies in rat models have shown that nitric oxide bioavailability is reduced during healthy ageing owing to its sequestration by reactive oxygen species (ROS)[8]. However, atherosclerosis and arteriosclerosis promote production of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase (eNOS), thereby decreasing endothelial nitric oxide production and further reducing its bioavailability. This effect contributes to impairment of cerebrovascular autoregulation[9], as nitric oxide relaxes the smooth muscle cells responsible for cerebrovascular autoregulation, which maintains cerebral blood flow through adjustments of vascular tone in response to alterations in arterial pressure[10]. The reduction in autoregulation, along with the perivascular oedema caused by influx of albumin, which will be discussed later (see 'Blood–brain barrier breakdown'), increases expression of adhesion receptors, including P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which contribute to leucocyte and platelet adhesion[11]. Leucocytes migrate into the perivascular space, helping lymphocytes and other inflammatory cells to pass through the glial basement membrane and migrate from the perivascular space into the brain parenchyma[6]. Platelet adhesion leads to thrombus formation (see 'Clotting pathway dysfunction')[12]. Studies in mice have shown that the deficiency of nitric oxide also reduces nitrosylation of neuronal calpain, thereby increasing calpain activity, which activates cyclin-dependent kinase and results in tau phosphorylation[13,14]. Accumulation of hyperphosphorylated tau, which is a key feature of AD, has been linked to VCID[14]. Importantly, endothelium-dependent vasodilation varies depending on vessel size: nitric oxide mainly mediates vasodilation of relatively large conduit vessels, whereas endothelium-dependent hyperpolarization is responsible for vasodilation of small resistance vessels[15].

Blood–brain barrier breakdown. The BBB is formed by the neurovascular unit (NVU), which consists of endothelial cells, pericytes (in capillaries), smooth muscle cells (in arterioles and venules), neurons, astrocyte end-feet, microglia, oligodendrocytes and the extracellular matrix[16]. The function of the BBB is to protect neurons from harmful factors present in the systemic circulation and to maintain the internal environment[17].

Multiple factors can contribute to the breakdown of the BBB. Endothelial dysfunction and pericyte degeneration that occur as a result of chronic hypoperfusion cause BBB impairment[18,19]. Upregulation of matrix metalloproteinase (MMP)-2, MMP-3 and MMP-9 (see 'Inflammation') has been associated with disruption of the basement membrane and loss of the tight junction proteins occludin, claudin-1, claudin-3, claudin-5 and claudin-12, thereby aggravating

BBB breakdown[17,20–23]. BBB breakdown facilitates red blood cell extravasation, which contributes to oxidative stress, entry of neurotoxic blood-derived products and inflammatory factors, and cellular infiltration. Other neurotoxic blood-derived proteins, such as albumin, fibrinogen, plasminogen, thrombin and low-density lipoprotein (LDL) cholesterol can also invade[17]. The influx of albumin causes perivascular oedema and impedes cerebral microcirculation and blood flow[17]. Other factors can also facilitate BBB breakdown, such as cytokines, leucocyte adhesion molecules, chemokines, growth factors and lipids, which mediate atherogenesis and platelet aggregation[17].

Oxidative stress. Fibrinogen extravasation caused by BBB breakdown (see 'Blood–brain barrier breakdown') leads to activation of microglia and astrocytes through integrins (CD11b and CD18) and Toll-like receptors that activate nuclear factor κB (NF-κB)-dependent transcription, ultimately leading to production of ROS[6,24]. Oxidative stress impairs endothelial nitric oxide signalling[25,26], switching nitric oxide production to superoxide production, which further increases ROS production, thereby accelerating oxidative stress and decreasing the anti-inflammatory effect of nitric oxide[27,28]. Extravasation of red blood cells caused by BBB breakdown (see 'Blood–brain barrier breakdown') releases neurotoxic Fe^{2+}, also leading to production of ROS[17].

Inflammation. Activated microglia led by fibrinogen extravasation (see 'Oxidative stress') produce hypoxia-inducible factor 1α, leading to formation of furin that activates MMP-2, MMP-3 and MMP-9 (ref. 20), which are involved in breakdown of the BBB (see 'Blood–brain barrier breakdown'). They also activate receptors for advanced glycation end-products (RAGE). When RAGE and advanced glycation end-products interact, ROS are generated through activation of nicotinamide adenine dinucleotide phosphate-oxidase, which activates NF-κB. NF-κB activates cytokine genes such as tumour necrosis factor (TNF), interleukin (IL)-1, IL-6 and IL-8 (ref. 29).

Activated microglia polarize into two broad phenotypes: pro-inflammatory and anti-inflammatory[30]. Pro-inflammatory microglia are induced by interferon-γ, whereas anti-inflammatory microglia are induced by IL-4 and IL-13 (ref. 24). Pro-inflammatory microglia mediate the neuroinflammatory cascade, releasing nitric oxide, TNF, IL-6 and IL-1β, which lead to neuronal damage[24]. TNF and IL-1β also act on astrocytes to induce secondary inflammatory responses[24]. Anti-inflammatory microglia release IL-10, IL-33, TGF-β, insulin-like growth factor-1 (IGF-1), nerve-derived growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which have neuroprotective effects[31].

Activated astroglia also have two broad polarized phenotypes, known as A1 and A2 astroglia[32]. A1 astroglia produce ROS, IL-1β, TNF and nitric oxide, which are neuroinflammatory and cause neuronal damage. A2 astroglia mediate a neuroprotective cascade, release BDNF, which inhibits RAGE, and vascular endothelial growth factor (VEGF)[32], which promotes angiogenesis, increases glucose supply to the brain and activates antioxidants, all of which indirectly result in neuroprotection.

Clotting pathway dysfunction. Upon endothelial damage induced by ageing or hypertension, subendothelial matrix proteins are exposed to the bloodstream, resulting in platelet adhesion and accumulation of collagen, a process mediated by endothelial von Willebrand factor (vWF)[12]. A coagulation cascade is then initiated, involving various coagulation factors and tissue factors, culminating in thrombin-mediated conversion of fibrinogen to fibrin, leading to thrombus formation and microvascular occlusion that contributes to the development of lacunes[12]. Thrombin also has direct neurotoxic effects, causing neuronal cell death, astrocytic proliferation and microglial activation via protease-activated receptor-1[33]. In response to thrombosis, stable fibrin chains are degraded by plasmin, which originates from circulating plasminogen, into D-dimer[34]. Plasmin also enzymatically degrades the neuronal matrix protein laminin. When the interaction between neurons and the neuronal matrix is disrupted, hippocampal neurons are sensitized to cell death[35].

Degeneration of neurons and glia. Several of the processes discussed in the previous sections lead to damage and death of neurons and glia. Inflammatory cytokines released from activated microglia and astrocytes directly cause neuronal damage[20]. MMP-2 and MMP-3 damage oligodendrocytes, thereby causing loss of trophic support to neurons and trophic uncoupling that leads to neuronal degeneration[6,20,36]. Furthermore, oxidative stress prevents endothelial trophic factors from supporting oligodendrocyte precursor cells, affecting oligodendrocyte production[37,38]. These processes result in myelin breakdown and white matter damage, which lead to release of myelin basic protein and neurofilament light chain (NfL)[20]. Vessel wall fibrosis, which is caused by extracellular matrix remodelling, and cellular oedema also contribute to white matter change, visible on MRI scans as white matter hyperintensities (WMHs), which causes

Box 1

Terminology

Various terms are used to refer to cognitive impairment and dementia associated with vascular pathology. In this Review, we use the term vascular cognitive impairment and dementia as it reflects the full clinicopathological continuum. However, various terms are used to refer to VCID in different diagnostic criteria, as follows:

- Vascular dementia (Diagnostic and Statistical Manual of Mental Disorders (DSM) 4 (ref. 237), International Classification of Disease 10 (ref. 238), National Institute of Neurological Disorders and Stroke Association Internationale pour la Recherche et l'Enseignement en Neurosciences[233])
- Ischaemic vascular dementia (Alzheimer's Disease Diagnostic and Treatment Centers criteria[239])
- Vascular cognitive impairment (American Heart Association and American Stroke Association[1], Vascular Impairment of Cognition Classification Consensus 2 criteria[235])
- Vascular cognitive disorders (Vascular Behavioural and Cognitive Disorders criteria[234])
- Major vascular neurocognitive disorder (DSM-5 (ref. 240))

Other terms are used to refer to sub-categories of VCID, such as post-stroke dementia, multi-infarct dementia, subcortical vascular dementia and subcortical ischaemic vascular disease and dementia (SIVD)[233,235,241] (Table 1). These terms represent putative pathophysiological processes.

Table 1 | Subtypes of vascular cognitive impairment and associated stroke subtypes and vascular lesions

VCID subtype NINDS-AIREN criteria[233]	VASCOG criteria[234]	VICCS-2 criteria[235]	Associated stroke subtype[a]	Associated vascular lesions
Multi-infarct dementia	Large vessel or atherothromboembolic disease	Multi-infarct dementia	Large-artery atherosclerosis	Large vessel disease
			Cardioembolism	Cardiac
Strategic single-infarct dementia	Large vessel or atherothromboembolic disease	Post-stroke dementia	Large-artery atherosclerosis	Large vessel disease
			Small-vessel occlusion	Small vessel disease
			Cardioembolism	Cardiac
Small-vessel disease with dementia (multiple lacunar strokes or Binswanger disease)	Small vessel disease	Subcortical ischaemic vascular dementia	Small-vessel occlusion	Small vessel disease
Hypoperfusion	Hypoperfusion	NA	Large-artery atherosclerosis	Large vessel disease
			Cardioembolism	Cardiac
Haemorrhagic dementia	Haemorrhage	Post-stroke dementia	Intracerebral haemorrhage	Small vessel disease
			Subarachnoid haemorrhage	Others
			Chronic subdural haematoma	

NINDS-AIREN, National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences; TOAST, trial of ORG 10172 in acute stroke treatment; VASCOG, Vascular Behavioural and Cognitive Disorders; VCID, vascular cognitive impairment and dementia; VICCS, Vascular Impairment of Cognition Classification Consensus. [a]TOAST classification used for ischaemic stroke[236].

a disconnection syndrome that manifests as executive impairment and psychomotor retardation[20,39].

Large vessel disease

LVD, which is also referred to as larger-artery atheromatous disease[40], is a collective term for pathological changes in medium (main visceral arteries and their initial branches[41]) and large arteries (aorta and its major branches[41]) that contribute to atherosclerosis and atherothrombosis followed by the rupture of atheromatous plaques. These changes differ from arterial stiffening that occurs with age and independently of atherosclerosis, which reflects progressive fragmentation and loss of elastin fibres and accumulation of stiffer collagen fibres in the tunica media of large vessels[42]. Many pathophysiological processes involved in LVD are the same as those discussed in the previous sections for SVD. However, additional pathophysiological processes occur in larger vessels owing to the fact that the number of smooth muscle cells is greater in vessels with larger diameters[43] and these smooth muscle cells produce elastin and collagen that are involved in plaque formation. This plaque formation starts when LDL cholesterol that has been deposited in the arterial intima is modified by ROS into oxidized LDL (oxLDL), which is phagocytosed by macrophages to form foam cells, which migrate to the intima and interact with collagen, elastin and elastase to form lipid plaques and thicken the vessel wall[44]. Continuous narrowing of the lumen is caused by a gradually expanding atheromatous plaque, leading to vessel stenosis and a consequent reduction in cerebral blood flow[45].

The pathology of atherosclerosis differs between intracranial and extracranial vessels. Intracranial arteries are muscular arteries with fewer elastic fibres, such as elastin, than extracranial arteries[46]. In intracranial atherosclerosis, collagen tissue replaces the muscle fibres in the tunica media and the lesion develops mainly as fibrous plaques with fewer lipids[47]. Intracranial atherosclerosis has a later onset than extracranial atherosclerosis, increasing rapidly in the sixth decade of life[48]. Intracranial atherosclerosis is associated with increased levels and activity of MMP-9 owing to age-related reductions in antioxidant enzyme activity and increased oxidative stress from hypertension, diabetes and the metabolic syndrome[47,49].

Cerebral cardioembolism

Cerebral cardioembolism is caused by occlusion of cerebral blood vessels by thrombi from the heart as a result of several cardiac conditions, of which atrial fibrillation is the most prominent. In atrial fibrillation, congestion in the left atrium reduces forward blood flow, leading to thrombus formation[50]. Other factors that tend to contribute to thrombus formation are endothelial dysfunction in the heart that results in decreased production of nitric oxide, elevated levels of vWF and activation of P-selectin. Adhesion of platelets and neutrophils to these adhesion molecules also leads to thrombus formation[51].

According to a conceptual framework known as the atrial substrate model, which was developed in 2016, atrial cardiomyopathy is an important root cause of systemic embolism, including cerebral embolism[52]. The ageing process and various vascular risk factors are thought to give rise to aberrations and remodelling of the atrial tissue, referred to as atrial cardiomyopathy, ultimately leading to the onset of atrial fibrillation and thromboembolism. This comprehensive model has important implications for stroke prevention; knowledge that atrial fibrillation can occur as a result of vascular risk factors and arrhythmia suggests a new target for stroke prevention strategies in people who are susceptible to thromboembolism[52].

Intracranial haemorrhage

Intracerebral haemorrhage. Primary intracerebral haemorrhage involves the extravasation of blood into the brain parenchyma owing to the rupture of vessels associated with lipohyalinosis or microaneurysms, although the pathogenic mechanisms are not fully understood[53]. Haemorrhage in cortical region and that in subcortical region are thought to have different aetiologies, with some overlap. Haemorrhage from small vessels in the subcortical regions is thought to result from

the effects of ageing and hypertension, and it might arise from dysregulation of MMPs, elastin and collagen and from increased levels of inflammatory cytokines[53]. Haemorrhage in the cortical regions is often associated with cerebral amyloid angiopathy[54,55], which is characterized by Aβ deposition in cortical and leptomeningeal vessels that leads to degeneration of smooth muscle cells[56]. Some evidence suggests that Aβ also damages pericytes and leads to increased vascular permeability[53].

Subarachnoid haemorrhage. Spontaneous subarachnoid haemorrhage (SAH) usually results from the rupture of a cerebral aneurysm, leading to haemorrhage and thrombus formation. Various cascades related to microglia-associated and astrocyte-associated immunoregulation and inflammatory responses are involved in the pathogenesis of SAH and brain injury after SAH[57]. Cerebral aneurysms are cystic lesions that form mainly in bifurcations of blood vessels where shear stress is high. High shear stress activates NF-κB-dependent induction of monocyte chemoattractant protein-1 expression, which can induce macrophage infiltration into the vessel wall, triggering an inflammatory response contributing to the formation and enlargement of cerebral aneurysms[58,59]. Given that many drugs can inhibit NF-κB, the NF-κB cascade is expected to be an important target in the management of aneurysms[58].

Brain injury that results from subarachnoid haemorrhage is broadly classified into early brain injury, which occurs within 72 h after SAH onset, and delayed cerebral ischaemia, which commonly occurs 3–14 days after SAH onset[60]. However, the distinction between these categories is not clear. When arterial blood extravasates into the subarachnoid space upon the rupture of an aneurysm, intracranial pressure increases and cerebral perfusion pressure declines rapidly, leading to widespread cerebral ischaemia and subsequent cerebral oedema. Early cerebral vasospasm also has a role in cerebral ischaemia, as vascular disruption exposes tissue factors and causes microthrombus formation in the cerebral vasculature. Moreover, the presence of haematomas in the subarachnoid space triggers inflammatory cascades involving substances such as IL-1β, IL-6 and TNF. The disruption of the BBB results in brain oedema and, ultimately, neuronal apoptosis. In addition to the above, cortical spreading depolarization, which involves sustained depolarization of neurons, results in vasoconstriction leading to reduced cerebral blood flow and oxygen supply[61]. Furthermore, the disruption of brain autoregulation can reduce blood flow and increase the risk of cerebral ischaemia[61].

Molecular biomarkers in VCID

Numerous biomarkers could be associated with the pathophysiological pathways described in the previous sections, and some of these have been investigated in the context of VCID. In the sections that follow, we discuss possible biomarkers of VCID, focusing on those that have been empirically examined in VCID. We group the biomarkers according to their molecular type.

In addition, we have considered the potential of VCID biomarkers identified within the biomarker framework proposed by the National Institutes of Health National Centre for Advancing Translational Sciences (NIH-NCATS)[62] (Supplementary Tables 1–4). We divide potential biomarkers into four categories according to their potential uses: diagnostic, for those with potential for diagnosis of VCID or its differential diagnosis from AD; risk, for those associated with imaging findings, such as WMHs, cerebral microbleeds and lacunes, that are surrogate markers of SVD, which increases the risk of VCID; monitoring or prognostic, for those that could be used to assess the likelihood of disease progression; and pharmacodynamic, for those that could be used as surrogate clinical outcomes in clinical trials.

Proteins and peptides

A large number of proteins and peptides have been investigated for their potential as biomarkers of VCID (Supplementary Table 1). These protein candidate biomarkers are associated with various pathological processes involved in VCID, but evidence for their association with VCID is mixed.

Endothelial dysfunction. Biomarkers of endothelial dysfunction, such as cell adhesion molecules, selectins and ADMA, are elevated in many vascular diseases and lack brain specificity. As a result, the consistency and reproducibility of the findings in the context of VCID are low.

In the Framingham Heart Study, serum levels of ICAM-1 were positively correlated with WMH volume and the prevalence of silent brain infarcts (SBIs)[63], suggesting an association with the risk of VCID. In another study, serum levels of VCAM-1 were associated with the risk of VCID[64] and WMHs[65]. However, in the Rotterdam Study, no relationship was observed between serum levels of ICAM-1 or VCAM-1 and the risk of VCID[66]. In a case–control study, plasma levels of P-selectin did not differ significantly between individuals with lacunes and healthy controls[67]. However, in another study, plasma levels of E-selectin were higher among people with severe cerebrovascular disease than among healthy controls[64].

Evidence suggests that plasma levels of ADMA are elevated in people with SVD[68], and high plasma levels were associated with a higher prevalence of SBIs in the Framingham Offspring Study[69] and with lacunes and WMHs in other studies[70]. Furthermore, one study has shown that the ratio of arginine to ADMA was lower among people with lacunes or WMHs than among those without[71], which is important given that ADMA competitively inhibits arginine binding to endothelial nitric oxide.

Some biomarkers of endothelial dysfunction, such as adrenomedullin and placental growth factor (PlGF), exhibit greater brain specificity and, therefore, have greater potential in VCID. Adrenomedullin is primarily secreted by endothelial cells in the brain in response to ischaemia and exhibits multiple physiological functions, including anti-inflammation, vasodilation and angiogenesis[72,73]. A longitudinal study of 288 people without dementia and with no history of stroke revealed that serum levels of mid-regional pro-adrenomedullin, which is a stable fragment of the adrenomedullin precursor, increased significantly in association with progression of subcortical WMHs[74]. Moreover, these levels were inversely correlated with scores on tests of cognitive function[74].

PlGF interacts with VEGF to promote angiogenesis and regulate vascular permeability[75,76]. PlGF has an important role in the intricate process of cerebral circulation development[77] but the mechanisms behind its role in this development are not completely known. Levels of PlGF increase markedly in response to cerebral ischaemia, indicating promise as a biomarker of endothelial dysfunction. Indeed, the MarkVCID consortium demonstrated that high plasma levels of PlGF were associated with WMH severity and functional cognitive decline[76], and in another study, plasma and cerebrospinal fluid (CSF) levels of PlGF were higher among people with SIVD than among healthy controls[78].

BBB breakdown. One promising biomarker of BBB breakdown is soluble platelet-derived growth factor receptor β (sPDGFRβ), which is released into the CSF upon pericyte degeneration. In murine models

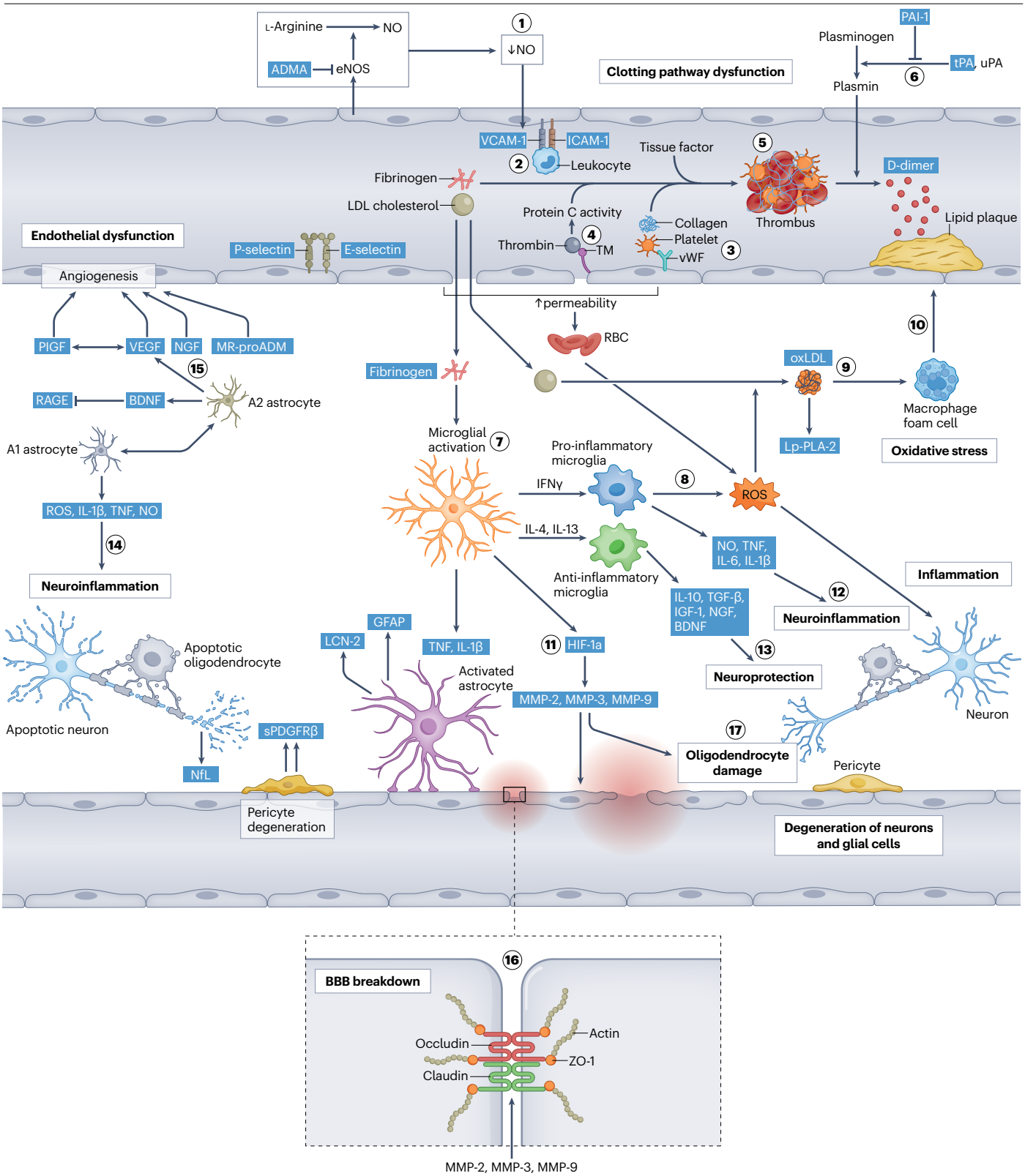
L-Arginine
NO
ADMA
eNOS
1
↓NO
Clotting pathway dysfunction
PAI-1
Plasminogen
tPA, uPA
6
Plasmin
VCAM-1
ICAM-1
2
Leukocyte
Tissue factor
5
Thrombus
D-dimer
Fibrinogen
LDL cholesterol
Protein C activity
Thrombin
4
TM
Collagen
Platelet
vWF
3
Lipid plaque
Endothelial dysfunction
P-selectin
E-selectin
Angiogenesis
↑permeability
RBC
PIGF
VEGF
NGF
MR-proADM
15
RAGE
BDNF
A2 astrocyte
A1 astrocyte
Fibrinogen
oxLDL
9
10
Macrophage foam cell
Lp-PLA-2
Oxidative stress
Microglial activation
7
Pro-inflammatory microglia
IFNγ
8
ROS
IL-4, IL-13
Anti-inflammatory microglia
NO, TNF, IL-6, IL-1β
Inflammation
ROS, IL-1β, TNF, NO
14
Neuroinflammation
IL-10, TGF-β, IGF-1, NGF, BDNF
12
Neuroinflammation
GFAP
LCN-2
TNF, IL-1β
11
HIF-1a
13
Neuroprotection
Apoptotic oligodendrocyte
Activated astrocyte
MMP-2, MMP-3, MMP-9
Neuron
17
Oligodendrocyte damage
Apoptotic neuron
sPDGFRβ
NfL
Pericyte
Pericyte degeneration
Degeneration of neurons and glial cells
16
BBB breakdown
Occludin
Actin
ZO-1
Claudin
MMP-2, MMP-3, MMP-9

Fig. 1 | Pathophysiological processes of vascular cognitive impairment and dementia, their interactions and potential biomarkers. Multiple pathophysiological processes have been implicated in vascular cognitive impairment and dementia, and these processes interact with one another. The main processes are as follows. Endothelial dysfunction: atherosclerosis and arteriolosclerosis increase production of asymmetric dimethylarginine (ADMA), which inhibits endothelial nitric oxide synthase (eNOS), thereby reducing endothelial nitric oxide (NO) production (1) and contributing to dysfunction of vascular autoregulation. Consequent hypoperfusion and hypoxia lead to increased expression of adhesion receptors, including selectins and cell adhesion molecules (2), which contribute to increased vascular permeability. Clotting pathway: platelets adhere to the vessel wall via von Willebrand factor (vWF), then to collagen, leading to activation and adhesion of platelets (3). Thrombomodulin (TM) binds to thrombin (4), leading to activity of protein C which regulates coagulative pathways. Tissue factor initiates a coagulation reaction to form a thrombus (5), leading to microvascular occlusion. Activity of urokinase plasminogen activators (uPAs) and tissue plasminogen activators (tPAs) normally leads to breakdown of thrombi via production of plasmin (6), but plasminogen activator inhibitor-1 (PAI-1) inhibits their activity. Oxidative stress: extravasation of fibrinogen results in activation of microglia (7), and pro-inflammatory microglia produce reactive oxygen species (ROS). Extravasation of red blood cells also leads to production of ROS owing to perivascular accumulation of iron-containing proteins that release Fe^{2+} as they are broken down. ROS oxidize LDL to form oxidized LDL (oxLDL) (8). oxLDL activates lipoprotein-associated phospholipase A2 (Lp-PLA2), which further promotes expression of adhesion molecules (2) and is phagocytosed by activated macrophages to form foam cells (9), which contribute to formation of lipid plaques (10). Inflammation: activated microglia produce HIF-1α (11), which in turn activates matrix metalloproteinase (MMP)-2, MMP-3 and MMP-9, contributing to blood–brain barrier (BBB) breakdown. BBB breakdown facilitates extravasation of red blood cell and other neurotoxic blood-derived proteins, such as fibrinogen. Pro-inflammatory microglia mediate a neuroinflammatory cascade, leading to neuronal damage and death (12). Anti-inflammatory microglia mediate a neuroprotective cascade (13). A1 astroglia mediate the neuroinflammatory cascade (14), whereas A2 astroglia mediate the neuroprotective cascade (15). Breakdown of the BBB: endothelial dysfunction and pericyte degeneration owing to chronic hypoperfusion cause BBB destruction. MMP-2, MMP-3 and MMP-9 disrupt the tight junction proteins occludin and claudins (16), resulting in tight junction permeability in the vascular endothelium. Degeneration of neurons and glial cells: inflammatory cytokines directly cause neuronal damage (12 and 14), and MMP-2 and MMP-3 induce oligodendrocyte damage (17), causing loss of trophic support to neurons and trophic uncoupling. These processes lead to neuronal cell death. BDNF, brain-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; ICAM-1, intercellular adhesion molecule-1; IGF-1, insulin-like growth factor-1; LCN-2, lipocalin-2; MR-proADM, mid-regional pro-adrenomedullin; NfL, neurofilament light chain; NGF, nerve growth factor; PlGF, plasma growth factor; RAGE, receptors for advanced glycation end-products; RBC, red blood cell; sPDGFRβ, soluble platelet-derived growth factor receptor-β; TGF-β, transforming growth factor-β; TNF, tumour necrosis factor; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; ZO-1, zonula occludens-1.

and in humans, sPDGFRβ is cleaved from pericytes and its CSF levels become elevated when pericytes are injured[79–81]. CSF levels of sPDGFRβ are also increased in humans during the initial stages of cognitive impairment, and dynamic contrast-enhanced MRI in patients with mild cognitive impairment (MCI) has shown that the increase correlates with BBB breakdown in the hippocampus regardless of whether patients have alterations in Aβ and/or tau biomarkers[18,81–83]. These findings suggest that detection of pericyte injury and BBB breakdown through CSF levels of sPDGFRβ could be an early indicator of VCID[18,81].

MMPs and the ratio of MMPs in the CSF and serum also hold promise as biomarker of BBB breakdown and have been studied extensively in the context of VCID, but studies of blood or CSF alone have produced inconsistent results. In a study of plasma and CSF levels of MMP-2, MMP-3 and MMP-9 in people with SIVD or AD[78], CSF levels of MMP-2 were higher among people with SIVD than among healthy controls, though levels of the other MMPs did not differ between the groups[78]. In a comparison of people with SIVD or AD and healthy controls, CSF levels of MMP-3 did not differ significantly[78,84]. In this and another study, CSF levels of MMP-9 were higher among patients with VCID or SIVD than among healthy controls or people with AD[84,85], whereas levels were similar between these groups in another study[78].

Some evidence suggests that measuring the ratio of MMPs in the CSF to that in the serum or the plasma — known as the MMP index — could improve the diagnostic value of MMPs, as baseline blood concentrations of different MMPs vary. In a study of people with VCID, the MMP-2 index was lower and activity of MMP-3 was higher among people with the condition than among healthy controls[86]. The MMP-2 index was negatively correlated with the CSF–serum albumin quotient, particularly in patients with SIVD[86]. The conclusion of this study was that the combination of MMP-2 index and MMP-3 activity could distinguish patients with SIVD from those with other forms of VCID with high specificity[86]. In another study, the MMP-2 index was higher among people with SIVD than among healthy controls[87]. Studies of MMPs and cognitive function have also demonstrated that plasma levels of MMP-2 and MMP-9 in people with VCID were correlated with frontal lobe dysfunction[78,88].

Inhibitors of MMPS, known as tissue inhibitors of metalloproteinases (TIMPs), could also have potential as biomarkers in VCID, though findings to date are inconsistent. In a study of people with SIVD or AD, CSF levels of TIMP-1 were higher among people with SIVD than among people with AD or healthy controls, though CSF levels of TIMP-2 did not differ between the groups[84]. In another study, CSF levels of neither TIMP-1 nor TIMP-2 differed between people with VCID, people with AD and healthy controls[85].

Vascular permeability that results from BBB breakdown can be assessed by measuring the CSF–serum albumin quotient[89], and this measure consistently differentiates VCID from AD. In a large cohort study, the CSF–serum albumin quotient was higher in VCID and mixed dementia than in AD[90]. Similarly, in the Gothenburg MCI Study, the CSF–serum albumin quotient was higher among people with VCID, particularly among those with subcortical SVD, than among people with AD[91].

Oxidative stress. Molecules associated with oxidative stress, such as oxLDL and lipoprotein-associated phospholipase A2 (Lp-PLA2), are biomarkers of advanced atherosclerosis rather than direct biomarkers of VCID. Nevertheless, the association between levels of these molecules and the risk of VCID has been investigated.

In one case–control study, groups with the highest plasma levels of oxLDL also had the highest incidences of VCID[92]. By contrast, in another study, no association was observed between elevated plasma levels of oxLDL and the risk of VCID[93]. High serum levels of Lp-PLA2 have been associated with an increased risk of dementia[94]. Both the Framingham Heart Study and the Northern Manhattan Study

demonstrated that high serum levels of Lp-PLA2 were associated with severe WMHs and SBIs[63,95].

Another molecule related to oxidative stress is malondialdehyde, a degradation product of membrane phospholipids peroxidized by ROS[96]. In one study, plasma MDA levels were higher in VCID than in AD[97], though another study has reported comparable levels in these two groups[98].

Inflammation. The involvement of multiple molecular pathways in neurovascular inflammation gives rise to several potential biomarkers. However, investigation of these candidate biomarkers in the context of VCID has not produced consistent results. This inconsistency makes use of inflammatory biomarkers in VCID challenging.

Studies of IL-6 levels for differential diagnosis have produced varied results. Some studies have shown that serum and CSF IL-6 levels were higher among people with VCID than among healthy controls[99,100]. However, other studies have indicated that serum and CSF levels of IL-6 are comparable in people with VCID, people with AD and healthy controls[78]. Similarly, some studies have suggested that serum levels of IL-6 were positively correlated with WMH severity[101] (assessed with Fazekas scales) and frontal lobe dysfunction[102], whereas a larger cohort study has found no association between plasma levels of IL-6 and WMHs[103] or risk of VCID[66].

A few studies of IL-1β have also produced mixed results. In one case–control study, plasma levels of IL-1β were higher among patients with VCID than among controls[104]. However, in other studies, serum and CSF levels of IL-1β in people with VCID did not differ from the levels in those with AD or healthy controls[105].

C-reactive protein (CRP) is commonly used as a specific marker of systemic inflammation, as it is synthesized in the liver in response to various inflammatory cytokines in vivo[106]. A case–control study and a small cross-sectional study have demonstrated an association between elevated serum or plasma levels of CRP or high-sensitivity CRP and VCID risk[66,99,107]. However, other case–control studies have reported no significant differences[92,108]. High serum levels of CRP were associated with WMHs in the Three-City Study[110] and the Rotterdam Scan Study[111] and with lacunes in a cross-sectional study[112], though another research study has reported no relationship between CRP levels and WMHs[113]. Clinically, high serum levels of CRP have also been associated with lower scores in Frontal Assessment Battery cognitive tests[102] and with impaired performance in tests of verbal fluency[114].

TNF selectively induces oligodendroglial cell death via activation of TNF receptor 1 (p55) and caspase 2 or caspase 3 (ref. 115). Several studies have consistently shown that plasma levels of TNF were higher among patients with VCID, especially SIVD, than among healthy controls[78,104,116].

The VEGF family are master regulators of angiogenesis and vascular permeability in development and diseases[117], and the Gothenburg MCI study has shown that people with VCID had significantly higher CSF levels of VEGF than healthy controls[118]. However, another study has reported that CSF levels of VEGF did not differ between people with VCID, people with AD and healthy controls[119]. Specific subtypes of VEGF have also been studied in the context of VCID. In a large population-based cohort, serum levels of VEGF-A, which is predominantly associated with angiogenesis[120], were associated with VCID[121] and were significantly higher among people with VCID than among healthy controls[78]. Moreover, plasma levels of VEGF-C, which is associated with lymphangiogenesis[120], were higher among people with SIVD than among healthy controls, and CSF levels were higher among people with SIVD than among those with AD[78]. NGF also has a role in angiogenesis, as well as promoting neurotrophic effects in sympathetic neurons[122]. In one case–control study, people with VCID had lower CSF levels of NGF than healthy controls[109].

TGF-β has a crucial role in angiogenesis, neuroprotection, neuroimmune functions, neural regeneration and synaptic plasticity[123], and its levels seem to be associated with VCID. A case–control study demonstrated that plasma levels of TGF-β were higher among people with VCID than among people without dementia[124]. Similarly, another study showed that CSF levels of TGF-β were higher among patients with VCID than among healthy controls[118]. TGF-β also has an important role in genetic models of VCID, such as cerebral autosomal-recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) and *COL4A1*–*COL4A2*-related disorders (see 'Biomarkers in genetic models').

RAGE is a transmembrane receptor involved in the pro-inflammatory response[125], and soluble RAGE results from either alternative splicing or proteolytic cleavage of the full-length RAGE protein[126]. In one study of patients with acute stroke, decreased serum levels of soluble RAGE predicted cognitive impairment after stroke[127]. BDNF is an inhibitor of RAGE, and its effects reduce inflammation, protect neurons, increase synaptic activity and increase endothelial nitric oxide levels[128]. Given this involvement in several physiological processes involved in VCID, BDNF might be expected to have high potential as a biomarker. However, in a prospective study of patients with ischaemic stroke, plasma levels of BDNF at stroke onset were not related to the development of post-stroke dementia[129], and CSF levels of BDNF did not differ between people with VCID and healthy controls[109].

IGF-1 regulates phagocytosis of reactive astrocytes[130] and modulates cerebral microvascular injury and neuroinflammation[131]. In a prospective study of patients with MCI, low serum levels of IGF-1 were associated with a greater risk of developing VCID during the 3.6-year follow-up period[132]. Other studies have shown that serum levels of IGF-1 were lower among people with VCID than among healthy controls[133] and lower among people with SVD with cognitive impairment than among people with SVD without cognitive impairment[134].

Lipocalin-2 is highly expressed in the CNS in response to injury or inflammation, is mainly secreted by reactive astrocytes and promotes neuroinflammation[135]. In a neuropathology study, lipocalin-2 staining revealed higher immunoreactivity in brains from people with multi-infarct dementia than in brains from people with AD[136]. A study of four independent cohorts has shown that CSF levels of lipocalin-2 were higher in VCID than in AD and non-ischaemic neuropsychiatric diseases[136].

IL-18 is a central inflammatory marker that is expressed by activated microglia and activated astrocytes, has a role in central nervous system (CNS) development and is an upstream regulator of brain immune and inflammatory processes[137]. A combined study of the MarkVCID and ASPIRE cohorts revealed that a network of inflammatory molecules co-ordinated by serum IL-18 was associated with WMHs[138]. A case–control study has also shown that plasma levels of IL-18 were higher among people with VCID than among people without dementia[124].

Clotting pathway dysfunction. Disruptions to the clotting pathway are involved in the pathophysiological processes that underlie VCID, and some evidence suggests an association between hypercoagulable states and VCID[139]. Multiple molecules involved in the clotting pathway

could have potential as biomarkers in VCID, though the value of such markers in this context remains unclear[139].

One such potential biomarker is fibrinogen, a soluble plasma glycoprotein produced by the liver that is involved in the coagulation cascade[140]. A 17-year prospective cohort study and a case–control study have demonstrated an association between plasma levels of fibrinogen and VCID[108,141]. However, another cohort study did not find such an association[142]. A 2-year prospective cohort study has shown that plasma levels of fibrinogen were associated with lacunes and WMHs[143]. However, plasma levels of fibrinogen were not associated with WMHs, SBIs or cerebral microbleeds in the Framingham Heart Study[63] or with enlarged perivascular spaces in a cohort study[144]. Regardless of the associations, fibrinogen levels are elevated in diseases other than cerebrovascular diseases, including myocardial infarction and deep vein thrombosis[145], so it cannot be a biomarker for VCID alone.

D-dimer is produced during the breakdown of thrombi, and a 4-year cohort study demonstrated that elevated plasma levels of D-dimer were associated with an increased risk of VCID[142]. Furthermore, a case–control study has shown that plasma levels of D-dimer were higher among people with VCID than among people with AD[108] or healthy controls[146]. In particular, people with Binswanger disease had higher plasma levels of D-dimer than people without cerebrovascular disease[147,148].

Plasma levels of vWF also show promise as a biomarker of VCID. In one study, plasma levels of vWF were higher among people with VCID[146] and people with lacunes[67] than among healthy controls. Moreover, high plasma levels of vWF have been associated with a greater severity of WMHs according to the grading system used in the study[149].

Thrombomodulin is an endothelial integral membrane protein that binds to the procoagulant thrombin, and the thrombomodulin–thrombin complex activates the circulating zymogen protein C[150]. In a study of people with SVD, thrombomodulin immunoreactivity was higher among patients with SVD than among healthy controls, and this immunoreactivity was positively correlated with SVD severity[151].

Plasminogen activator inhibitor-1 (PAI-1) is the principal inhibitor of urokinase plasminogen activators and tissue plasminogen activators, both of which mediate the production of plasmin from plasminogen[152]. Plasmin cleaves cross-linked fibrin to produce fibrin degradation products[153]. High plasma levels of PAI-1 were associated with an increased risk of VCID in a 17-year prospective cohort study[141]. One case–control study has also shown that people with VCID had higher plasma levels of PAI-1 than healthy controls[146], though other studies have demonstrated similar levels in the two groups[133,154].

Neuronal and glial degeneration. Biomarkers of neuronal and glial degeneration include NfL and glial fibrillary acidic protein (GFAP). However, these proteins are elevated in all neurodegenerative diseases, so they cannot be used as biomarkers of VCID alone. Nevertheless, these markers have been associated with VCID and could, therefore, be useful in combination with other markers.

NfL has been extensively studied in relation to neuronal and glial degeneration. It is a component of the neuroaxonal cytoskeleton involved in various physiological processes, including axonal radial growth, neuroaxonal stability and electrical conductivity[155]. Elevated levels of NfL in the serum or plasma have been observed in people with VCID compared with healthy controls[156], and these levels have also been associated with WMHs, lacunes and cerebral microbleeds[157]. Similarly, CSF levels of NfL were higher among people with VCID than among healthy controls[158,159], and CSF levels also correlated positively with the extent of WMHs defined according to the Blennow–Wallin or Fazekas scales[159–161]. Serum levels of NfL have also been associated with processing speed in people with SVD[162]. Furthermore, in a longitudinal study of people with lacunar infarction and confluent WMHs (as defined in the Fazekas scale), baseline serum levels of NfL predicted cognitive decline and the risk of transition to dementia[163]. In this study, NfL levels did not change during the follow-up period.

GFAP is an astrocytic cytoskeletal protein. In pathological specimens of humans and mice, upregulation of GFAP is strongly associated with reactive astrocyte remodelling[164]. A cross-sectional analysis has demonstrated that the serum titre of GFAP antibodies was higher among people with VCID than among healthy controls[165]. Serum GFAP levels were also higher among a cohort of people with recent small subcortical infarcts than among healthy controls[166]. In another cohort of people with SVD, higher serum levels of GFAP were associated with worse neurocognitive functions[161].

Glossary

Cerebral microbleeds
Small areas of signal void with associated blooming on T2*-weighted MRI or susceptibility-weighted imaging.

Lacunes
Round or ovoid subcortical, fluid-filled cavities of 3–15 mm in diameter; the MRI signal is similar to that of cerebrospinal fluid.

Perivascular spaces
Fluid-filled spaces that surround blood vessels in the grey or white matter.

White matter hyperintensities
Abnormalities of variable size in the white matter that appear as hyperintensity without cavitation on T2-weighted MRI.

Metabolites as biomarkers

Several metabolites have been investigated for their potential as biomarkers of VCID (Supplementary Table 2). The most well-studied metabolite is homocysteine, which is an independent risk factor not only for cerebrovascular disease but also for coronary artery disease and peripheral arterial disease[167].

Homocysteine is a sulfur-containing amino acid that is produced during methionine metabolism[168]. Accumulation of homocysteine can arise as a result of genetic defects or a deficiency of vitamin B12 and folate[168]. Homocysteine is a potential marker of VCID as it is involved in the oxidative stress pathways that contribute to the pathophysiology — superoxide is produced by the reaction of homocysteine with Cu^{2+} and reduces cerebral blood flow by scavenging nitric oxide via the formation of peroxynitrite[169]. Elevated plasma levels of homocysteine induce early inflammatory changes in microglia and astrocytes[170], and studies have shown that plasma levels of homocysteine were higher among people with VCID than among people with AD[171] or among healthy controls[92,171–176]. Moreover, plasma levels of homocysteine have been associated with WMHs[177], SBIs[178] and cerebral microbleeds[179] and correlate negatively with functional and cognitive status[180].

Another metabolite that has been investigated is 24*S*-hydroxycholesterol, an oxidation product of cholesterol formed primarily in the brain[181]. One case–control study has demonstrated that people with VCID had higher circulating levels of 24*S*-hydroxycholesterol than people with depression and healthy controls[182]. By contrast, another case–control study has shown that circulating levels of 24*S*-hydroxycholesterol were lower among patients with VCID than

among healthy controls[183]. The discrepancy between these findings could be a result of the timing of blood sampling rather than inconsistency because 24*S*-hydroxycholesterol levels change with disease progression. In the early stages of VCID, 24*S*-hydroxycholesterol derives from degenerated neurons, and increased permeability of the BBB results in high blood levels[183]. However, as VCID progresses, the number of neurons that produce 24*S*-hydroxycholesterol decreases and blood levels of the metabolite reduce[183].

Lipids as biomarkers

Lipids have also been assessed as potential biomarkers in the context of endothelial dysfunction and inflammation in VCID (Supplementary Table 2). Results are inconsistent between atherosclerosis and cerebrovascular disease.

Concentrations of LDL cholesterol in the blood are positively correlated with the extent of atherosclerosis[184], but studies in the context of VCID and SVD have unexpectedly identified inverse associations. A combined analysis of data from the Three-City Study and the Epidemiology of Vascular Ageing Study revealed a negative correlation between LDL cholesterol concentrations and WMH volume[185]. Similarly, in a comparative study involving people diagnosed with subcortical small vessel dementia, AD or MD, concentrations of LDL cholesterol were lowest among those with subcortical small vessel dementia[186]. The underlying mechanisms are unclear. One speculative explanation is that cholesterol is vital for the development and sustenance of the CNS, so that lowering of cholesterol could hinder the ability to defend against and repair chronic white matter damage[186].

Ceramides are members of the sphingolipid family that regulate various cellular processes, including LDL aggregation, inflammation, endothelial dysfunction and cell proliferation, differentiation and apoptosis[187]. One study has shown that plasma levels of ceramide were lower among people with VCID than among healthy controls[188].

Circulating RNAs as biomarkers

MicroRNAs (miRNAs), which are short, non-coding RNAs that regulate gene expression, are the most commonly studied circulating RNAs, and several have been studied as candidate biomarkers of VCID (Supplementary Table 3). Two of the strongest candidates are miR-130b-3p, which modulates the polarization of pro-inflammatory macrophages mediated by interferon regulatory factor 1 and thereby has a role in regulating inflammation[189], and miR-10b-3p, which regulates *BDNF* expression[190]. Reduced plasma levels of these miRNAs enabled people with VCID to be distinguished from healthy controls, and this distinction was more accurate when the two were used in combination[191].

Other candidate biomarkers that modulate microglial activity are miR-146a and miR-222. miR146a promotes anti-inflammatory microglial polarization and, thereby, reduces microglial production of pro-inflammatory cytokines[192]. In one study, serum levels of miR-146a were elevated in VCID but decreased in AD[193]. miR-222 also acts on activated microglia and is associated with inflammation and apoptosis[194]. Levels of miR-222 in the CSF were higher among people with VCID than among healthy controls and people with AD, but the ability of this measure to discriminate between groups was low[158].

In addition to microglial miRNAs, miR-29a is highly expressed in astrocytes and targets the p53 upregulated modulator of apoptosis, maintains the uptake of glutamate in astrocytes, decreases oxidative stress and promotes neuronal survival[195]. In one study, serum levels of miR-29a enabled people with VCID to be distinguished from healthy controls[196]. In an independent study using a replication cohort, miR-29a was downregulated in patients with VCID. Furthermore, miR-29a expression was negatively correlated with Mini-Mental State Examination scores in patients with VCID[191].

Rather than measuring levels of individual miRNAs, combined measurements of many could provide greater sensitivity and specificity. Indeed, one risk prediction model based on supervised principal component analysis logistic regression was based on serum levels of 86 miRNAs[197]. This model enabled people with VCID to be distinguished from healthy controls with high accuracy[197].

MicroRNAs isolated from exosomes rather than freely circulating could also have greater potential as biomarkers. The contents of exosomes, which are a type of extracellular vesicle, reflect the state of the cells and tissues from which they derive, and exosomes can cross the BBB, making them potential sources of biomarkers of dementia[198]. In the context of VCID, exosomal miR-154-5p could be an important regulator of endothelial progenitor cell-mediated angiogenesis, and one study has shown that people with VCID had higher serum levels of this miR than healthy controls, enabling the groups to be distinguished with high accuracy[199]. Another exosomal miRNA, miR-223-3p, induces conversion of pro-inflammatory microglia into anti-inflammatory microglia and, in one study, was upregulated in patients with SVD compared with controls[200].

Biomarkers in genetic models

Genetic models of VCID are crucial for understanding the mechanisms that contribute to its development and are, therefore, ideal settings in which to look for candidate biomarkers. These genetic models include cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), CARASIL and *COL4A1*–*COL4A2*-related disorders.

CADASIL is an autosomal dominant inherited disease in which missense mutations in the *NOTCH3* gene cause accumulation of granular osmiophilic material in the tunica media of small blood vessels throughout the body, resulting in SIVD, in which neurological symptoms manifest at approximately 40 years of age[201]. An estimated 0.2% of the general population carry a *NOTCH3* mutation and consequently have a high susceptibility to stroke and vascular dementia[202]. The consequent necessity for early detection of CADASIL has driven biomarker research[202]. Several biomarkers of CADASIL have been identified. Plasma or serum levels of NfL were higher among people with CADASIL than among healthy controls[203] and also correlated with disease severity, disease progression and 17-year survival[204]. Biomarkers that can distinguish patients with CADASIL from healthy cohorts include plasma levels of vWF[205] and ADMA[206]. Moreover, in a mouse model of CADASIL, plasma levels of collagen α1(XVIII) and its cleavage product endostatin were increased, and levels of the Notch 3 extracellular domain were decreased[207,208].

Other genetic models of VCID — CARASIL and *COL4A1*–*COL4A2*-related disorders — emphasize the potential of TGF-β as a biomarker of VCID pathology. CARASIL is characterized by development of progressive WMHs, multiple lacunar infarcts, alopecia and osteoarthritis. The vascular pathology resembles that of severe solitary VCID: endothelial cell proliferation, loss of the smooth muscle cell layer and internal elastic rupture, without the accumulation of aberrant structures that occurs in CADASIL. The condition is caused by mutation of *HTRA1*, the product of which is a protease that suppresses TGF-β signalling[209]. The mutation consequently results in increased TGF-β secretion and signalling[209].

The *COL4A1* and *COL4A2* genes that are mutated in *COL4A1*–*COL4A2*-related disorders encode collagen α1(IV) and collagen α2(IV),

respectively, which are extracellular proteins that form the basement membrane. Pathological mutations in these genes lead to intracerebral haemorrhage, lacunes, cerebral microbleeds and WMHs[209]. Specific *COL4A1*-related disorders include hereditary angiopathy with nephropathy, aneurysms and muscle cramps syndrome, pontine autosomal dominant microangiopathy with leukoencephalopathy and hereditary multi-infarct dementia in a specific Swedish population[209]. Studies of mice with mutations in *COL4A1* have revealed that increased TGF-β signalling is linked to developmental abnormalities of angiogenesis within the CNS[210]. These irregularities are characterized by an increase in wall cells with anomalous morphology, altered expression of contractile proteins within vascular smooth muscle cells and age-related loss of arteriolar vascular smooth muscle cells[210].

Biomarker panels

Some of the potential VCID biomarkers that have been studied — particularly those related to inflammation and oxidative stress — are likely to be common to several age-related diseases. In addition, biomarker specificity is a challenge in multifactorial diseases such as VCID. One potential way to improve disease specificity is by combining several blood and CSF biomarkers to compensate for insufficiencies of individual biomarkers (Supplementary Table 4)[211], and some studies have been done to test proposed biomarker panels.

In the Gothenburg MCI Study, a longitudinal investigation indicated that combined CSF tau, $A\beta_{42}$ and NfL could predict the development of subcortical VCID in patients with MCI[212]. In this study, NfL predicted progression and the AD-specific markers were used to distinguish VCID from AD. The same study has also demonstrated that a biomarker panel including NfL, MMP-9, tau and $A\beta_{42}$ enabled people with AD to be differentiated from those with SIVD with a high degree of accuracy[84].

In a different study, a combination of biomarkers linked to systemic and vascular inflammation was more strongly associated with WMH progression and lacune development than each of the individual biomarkers alone[213]. In some studies, imaging markers have been added to blood and CSF biomarkers. For example, in one such study, the combination of BBB permeability measured with dynamic contrast-enhanced MRI and MMP-2 index distinguished patients with Binswanger disease from those with other types of VCID with high accuracy[214].

Artificial intelligence is an important emerging tool for determining disease-specific, optimized biomarker panels. Supervised and unsupervised machine learning have been used in some of the latest studies. For instance, use of supervised random forests to analyse multiplex assays of biomarkers in blood and CSF showed that MMPs, interleukins, angiopoietin 1 receptor and PlGF were important in diagnosis of mixed dementia[78]. Furthermore, agglomerative hierarchical clustering analysis, a type of unsupervised machine learning, enabled identification of subsets within a population of people with MCI owing to cerebrovascular disease on the basis of differences in levels of VEGF, MMP-1 and IL-8 (ref. 215).

Applications of biomarkers

The evidence discussed in the previous sections suggests that some molecules relevant to multiple pathways involved in the pathophysiology of VCID have potential as clinical biomarkers. However, various approaches have been taken in the studies of these biomarkers and their potential uses. For example, some have investigated the roles of biomarkers in differentiating people with VCID from healthy controls or people with other neurological disorders, particularly AD. Others, although fewer, have assessed the role of biomarkers in disease monitoring or clinical outcome. In the following sections, we integrate the findings and consider the evidence for various uses of molecular biomarkers in VCID.

Discriminating from healthy controls

Substantial evidence indicates that biomarkers of neuronal and glial degeneration, such as NfL and GFAP, can differentiate people with neurodegenerative diseases from healthy controls, but these markers cannot distinguish between diseases[156,158,159,165,166]. The same is true for many biomarkers related to endothelial dysfunction and inflammation — VCAM-1[64], ADMA[68], vWF[67,146], interleukins[99,104,124] and TNF[78,104,116] have all been proposed as diagnostic biomarkers of VCID but they are elevated in systemic diseases and cerebrovascular disease[216–219]. Biomarkers with high brain specificity, such as PlGF and MR-proADM, could offer more specific options. Indeed, in one study, PlGF enabled people with MCI and people with dementia and severe WMH to be distinguished from healthy controls with high accuracy[76]. Furthermore, inflammatory biomarkers in the CSF indicate increased permeability of the BBB, so they could help to differentiate people with VCID from healthy controls[109,118] — CSF levels of MMPs or MMP indexes could be useful in this regard[84–87].

Discriminating from Alzheimer disease

VCID and AD share many pathophysiological pathways, making them difficult to distinguish. A combination of NfL, $A\beta_{42}$ and tau protein has been found to distinguish VCID from AD with high accuracy[84,212]. Subcategorization of VCID has the potential to address more specific pathophysiology that could facilitate the distinction. For example, people with predominant subcortical SVD exhibit high levels of BBB permeability[220], and one study has shown that people with subcortical SVD had a higher CSF–serum albumin quotient than people with AD[90,91]. Other studies have shown that the MMP-2 index was higher among patients with SIVD than among healthy controls[86,87]. In addition, sPDGFRβ is indicative of pericyte injury and has emerged as a biomarker for early detection of BBB disruption[18,81], so it could have potential as a biomarker of SIVD.

In a study of four independent cohorts, lipocalin-2 was useful for differentiating between VCID and AD[136], suggesting that lipocalin-2 has an important role in the pathophysiology of VCID. Some evidence suggests that biomarkers involved in thrombus formation, such as fibrinogen and D-dimers, and plaque formation, such as oxLDL and Lp-PLA2, could also be useful for differentiating VCID from AD. One study has shown that levels of fibrinogen and D-dimer enabled VCID to be distinguished from AD[108], and another case–control study has reported that higher plasma concentrations of oxLDL were associated with a higher incidence of VCID[92].

Assessing risk, disease monitoring and prognosis

An association between white matter lesions and cognitive decline seems to be robust, but the cognitive domains affected seem to depend on the location of the lesion. For example, patients with multiple lacunar strokes and extensive WMHs have more prominent symptoms of frontal lobe dysfunction, including psychomotor slowing and executive dysfunction, owing to the involvement of frontal lobe white matter[221,222]. Blood levels of MR-proADM[74], IL-6[101,102], IL-18[102,138] and CRP[102,110,111,114] are associated with both WMH and frontal lobe dysfunction, suggesting these molecules could be useful biomarkers in VCID.

Biomarkers of endothelial dysfunction and inflammation are often associated with WMHs[63–65,70,71,74,76,101,110,111,138] and are also expected to be associated with cognitive dysfunction; as such dysfunction is associated with WMHs in the frontal lobe. Among these biomarkers, an inflammatory network co-ordinated by IL-18 could be particularly relevant to white matter injury that results from cerebrovascular disease. IL-18 is mainly produced by neurons within the brain but is also present in infiltrating immune cells after ischaemia[138]. Future studies of inflammatory biomarkers co-ordinated by IL-18 and their associations with cognitive function could, therefore, be informative.

Among metabolites, homocysteine is involved in inflammation, and high levels of it cause WMHs[177] and SBIs[178], ultimately leading to cognitive and functional impairments[179,180]. Homocysteine cannot be a diagnostic biomarker of VCID because it is also a cardiovascular risk factor[167], but it does have potential as a biomarker for measuring the risk and severity of VCID.

Blood or CSF levels of NfL are associated with WMHs[157,159–161], as is cognitive function[157,162,163]. NfL could, therefore, indicate neuroaxonal damage that is the ultimate outcome of pathology SVD.

Future investigation of the relationship between cognitive function and biomarkers could be facilitated by stratification of the six cognitive domains defined in the DSM-5 (ref. 223) into more complex categories. This stratification could enable research to be tailored to individual pathologies.

Clinical translation

Blood biomarkers are currently used as surrogate endpoints in clinical trials to assess outcomes and treatment efficacy, as peripheral blood samples are accessible, inexpensive and repeatable[224]. However, they are still rarely used as primary outcome measures, as they require clinical validation to determine whether they reflect the severity of the underlying pathology and/or the level of functional disturbance. They also need to be standardized and affordable.

As discussed in the previous sections, multiple associations have been identified between various molecular biomarkers and vascular lesions associated with VCID, but these associations have not yet translated into clinical applications. For example, even levels of NfL, which has been extensively studied as a marker of neuroaxonal degeneration[163], did not change over 3 years in people with SVD. Several difficulties underlie this lack of clinical translation.

First, the diagnostic and aetiological complexities inherent to VCID remain incompletely elucidated. None of the biomarkers alone captures the multifaceted pathologies that characterize VCID. In addition, healthy ageing induces alterations in the BBB, heightens reactive astrocyte activity and increases pericyte loss[225], thereby altering levels of many of the same molecules associated with VCID, so the pathophysiological processes involved in VCID are challenging to distinguish from physiological changes. Biomarker findings, therefore, need to be considered alongside clinical and cognitive evaluations to define diagnostic and prognostic parameters.

Second, development of biomarkers of VCID has not thus far been based on a clear framework for defining biomarkers, such as the NIH-NCATS biomarker framework[62]. Such a framework is needed to ensure that biomarkers appropriately reflect the biological processes and outcomes of a disease and that their use and context of use is clearly defined.

Third, biomarkers need to be clinically validated in diverse populations and research settings[226]. Clinical validation also requires standardized technical procedures and analytical approaches to determine sensitivity, specificity, precision and stability. However, sample sizes in VCID biomarker studies are often small, and reproducibility in independent cohorts has been low.

Finally, the ideal biomarker of VCID would need to meet multiple criteria (Box 2). Given the heterogeneous aetiologies of VCID, a single biomarker is unlikely to meet all these criteria, so a combination of biomarkers is likely to be necessary; however, identifying and validating a suitable panel poses its own challenges. Attempts so far have largely involved selection of multiple biomarkers from a single pathway, such as inflammation or BBB breakdown. This approach tends to increase sensitivity relative to the sensitivity of individual markers, but it does not tend to increase specificity. Arguably, a more useful approach is to select the most promising biomarkers from several pathways involved in VCID and combine them (Table 2). Of particular interest are combinations of blood and CSF biomarkers of VCID with molecular or imaging biomarkers of AD because AD is the most common differential diagnosis in an older person with cognitive impairment.

One approach to selection of biomarkers for a panel is to select on the basis of meta-analytical validation in each pathway. For example, a meta-analysis of inflammatory biomarkers in VCID has reported that blood level of IL-6 was a useful measure for differentiating VCID from AD[227]. Another approach is the use of genetic models of pure VCID, such as CADASIL. The development of biomarkers of SVD related to the pure VCID model will lead to a better understanding of the pathophysiology of SVD as a whole. However, CADASIL and other genetic models also have limitations, as they do not replicate all aspects of VCID pathogenesis, and some biomarkers, such as components of granular osmiophilic material, are specific to the models and cannot be translated into biomarkers for other types of VCID.

Machine learning has the potential to facilitate selection of biomarkers for panels. Use of approaches such as random forests[78] and hierarchical clustering analysis[215] with data extracted from blood and CSF assays and neuroimaging has already demonstrated their ability to improve identification of biomarkers. These approaches enable processing of large volumes of complex data and could, therefore, be useful for integrating the multiple aspects of VCID pathophysiology to

Box 2

The ideal biomarker

The ideal biomarker for vascular cognitive impairment and dementia would meet several criteria, as follows:

1. Reflect the pathophysiological processes involved in cerebrovascular disease and/or its impact on brain structure and function
2. Reflect the severity of the underlying pathology and/or the level of functional disturbance
3. Have high sensitivity and specificity for the disorder
4. Be safe, easy and cost-effective to measure
5. Show no major differences in associations between sexes and ethnicities
6. Strongly associate with a diagnosis of vascular cognitive impairment and dementia and/or its prognosis and/or the response to treatment

Table 2 | Promising biomarkers of vascular cognitive impairment and dementia that could form a diagnostic panel

Pathway	Biomarkers		
	Serum or plasma	CSF	CSF/serum ratio
Endothelial dysfunction	ICAM–1, VCAM-1, ADMA, MR-proADM and PlGF	PlGF	NA
BBB breakdown	MMP-2, MMP-3 and MMP-9	sPDGFRβ, MMP-2, MMP-3, MMP-9 and TIMP-1	Albumin, MMP-2, MMP-3 and MMP-9
Oxidative stress	oxLDL and Lp-PLA2	NA	NA
Inflammation	IL-6, IL-18, VEGF-A, VEGF-C and TGF-β	IL-6, VEGF-C, TGF-β and LCN2	NA
Clotting pathway dysfunction	Fibrinogen, D-dimer and vWF	NA	NA
Degeneration of neurons and glial cells	NfL and GFAP	NfL	NA

ADMA, asymmetric dimethylarginine; BBB, blood–brain barrier; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; ICAM, intercellular adhesion molecule; IL, interleukin; LCN2, lipocalin 2; Lp-PLA2, lipoprotein-associated phospholipase A2; MMP, matrix metalloproteinase; MR-proADM, mid-regional pro-adrenomedullin; NfL, neurofilament light chain; oxLDL, oxidized low-density lipoprotein; PlGF, placental growth factor; sPDGFRβ, soluble platelet-derived growth factor receptor-β; TGF, transforming growth factor; TIMP, tissue inhibitors of metalloproteinase; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.

determine the most informative combinations of biomarkers. Clinical applications of deep learning, in which artificial intelligence independently extracts features from training data and acquires rules and knowledge, are expected in the future and could further enhance biomarker identification and selection[228].

Future directions

Besides translation of VCID biomarker research into clinical application, research into these biomarkers can be taken in several novel directions. One example is the use of technological advances to identify biomarkers of increasingly specific processes. For instance, transcriptomics has revealed three distinct endothelial cell subtypes that exhibit phenotypic zoning along the arteriovenous axis, 11 vascular cell subtypes that include five subtypes of wall cells, and three subtypes of perivascular fibroblasts. This study reveals the potential for identifying specific biomarkers that are related to each cell type and, therefore, provide greater insight into specific, localized processes[229].

Biomarkers of vascular pathophysiology studied in the context of VCID could also become increasingly important in the context of AD treatment. Amyloid-related abnormalities, such as extravascular fluid and haemosiderosis, seem to occur in approximately 10% of patients who receive Aβ antibodies such as lecanemab[230]. These phenomena might result from vascular permeability following BBB breakdown[231], so biomarkers of the processes involved could be valuable in identifying these effects early.

In addition, extensive international collaborations and cross-ethnic approaches have the potential to increase our comprehension of disease susceptibility in all populations. Global, multi-omics biomarker investigations that consider genetic and environmental factors will not only advance our understanding of VCID pathophysiology but will also pave the way for novel therapeutic interventions[232].

Conclusions

Multiple potential biomarkers of VCID related to the pathophysiological pathways involved have been identified and could be used to facilitate biomarker-based diagnosis and subtyping of VCID. However, the associations identified have not translated into clinical application. To meet the challenge of biomarker-based diagnosis and facilitate the development of disease-specific interventions, the pace of biomarker development needs to accelerate. In particular, development should focus on the use of biomarker combinations and the application of machine learning to identify the optimal panels for diagnosis, prognosis and monitoring of treatment responses.

Published online: 13 November 2023

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Acknowledgements
The search strategies were peer reviewed by the UNSW librarian.

Author contributions
S.H. and P.S.S. conceived the idea of the Review. S.H. performed the literature search, prepared the original figures and tables, and wrote the first draft. G.K.H. and T.J. assisted with the literature search by assessing manuscripts against the review criteria. A.P., K.A.M., V.S.C., R.R., A.S., J.C.K., A.B., A.W., B.V.Z., M.I. and P.S.S. critically revised the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41582-023-00884-1.

Peer review information *Nature Reviews Neurology* thanks G. J. Biessels and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Review criteria We searched for papers using PubMed, Embase and Scopus with the terms ('vascular dementia' [Text Word]) OR ('Vascular cognitive impairment' [Text Word]) OR ('vascular contributions to cognitive impairment and dementia' [Text Word]) OR ('Vascular cognitive impairment and Dementia' [Text Word]) OR (Dementia, Vascular [Mesh]) OR (Cerebral Small Vessel Diseases [Mesh]) AND (Biomarker [Text Word] OR Biomarkers [Mesh] OR RNA [Text Word] OR RNA [Mesh]) AND (Plasma [Text Word] OR Serum [Mesh] OR Blood [Text Word] OR Cerebrospinal Fluid [Mesh] OR Circulating [Text Word]) on 16th December 2022. The reference lists of identified papers were used to identify additional papers on VCID biomarkers.