Biomarkers and Genomic Medicine (2015) 7, 43–56

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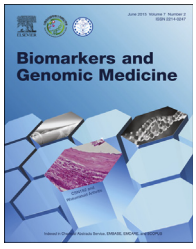

REVIEW ARTICLE

Biomarkers in vascular dementia: A recent update

Abhijeet Jagtap, Sonal Gawande, Sushil Sharma*

Saint James School of Medicine, Bonaire, Dutch Caribbean, The Netherlands

Received 30 May 2014; received in revised form 9 October 2014; accepted 14 November 2014
Available online 23 December 2014

KEYWORDS
cerebrospinal fluid;
Charnoly body;
neuroimaging;
serum

Abstract Vascular dementia (VaD) affects a broad spectrum of patients with various manifestations of cognitive decline, which could be attributed to cerebrovascular or cardiovascular disease. Diagnosis of VaD depends on the identification of environmental and genetic risk factors including; cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Mitochondrial oxidative stress, hypoxic ischemia, inflammation, accumulation of advanced glycation products, and proinflammatory cytokines have been implicated in the pathogenesis of VaD. Hence it is exceedingly important to determine the risk factors and molecular pathology by identifying specific biomarkers that can be broadly classified as: biochemical, molecular, genetic, endocrinological, anatomical, imaging, and neuropathological; for the early differential diagnosis, prognosis, and effective treatment of VaD. The biomarkers of VaD in the serum and cerebrospinal fluid samples include; phosphorylated tau, amyloid-β, matrix metalloproteases, sulfatids, albumin, and proinflammatory C-reactive proteins. In addition, Charnoly body (CB) formation and microRNAs can be detected as preapoptotic biomarkers of compromised mitochondrial bioenergetics to further confirm VaD. CB formation occurs in response to nutritional stress and/or neurotoxic insult in the most vulnerable hippocampal neurons due to cerebrovascular insufficiency, and can be attenuated by dietary interventions, physiological zinc supplementation, and metallothioneins (MTs). MTs provide ubiquinone-mediated neuroprotection by serving as free radical scavengers, by maintaining the mitochondrial redox balance, by inhibiting CB formation, and by inhibiting progressive neurodegenerative α-synucleinopathies. MTs also regulate zinc-mediated transcriptional activation of genes involved in cell growth, proliferation, and differentiation, and hence may be used as novel biomarkers of VaD. In addition to genetic analysis of MTs, Notch3, apolipoprotein E4, nitric oxide synthase, and cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy; omics and microRNA analyses may provide novel biomarkers of VaD. This review provides recent update on *in-vitro* biomarkers from the serum

* Corresponding author. Saint James School of Medicine, Plaza Juliana 4, Kralnedijk, Bonaire, Dutch Caribbean, The Netherlands.
E-mail address: Sharma@mail.sjsm.org (S. Sharma).

http://dx.doi.org/10.1016/j.bgm.2014.11.001

and cerebrospinal fluid samples and *in-vivo* neuroimaging biomarkers for the differential diagnosis and effective clinical management of VaD.

Introduction

Dementia may be defined as a progressive neurodegenerative disease characterized by loss of cognition, significant enough to cause functional disability in everyday life.[1,2] It is a major public health problem affecting over 20 million people around the world and the number is increasing exponentially in industrially-developed countries.[3] The prevalence of two major types of dementia, Alzheimer's disease (AD) and vascular dementia (VaD), is around 4.4% and 1—2 % respectively in industrially-developed countries; however, the prevalence is lower in developing countries.[4] AD accounts for 70—75% cases of dementia in elderly, whereas VaD comprises a small but significant group accounting to around 20% cases, the second most common form of dementia after AD.[4,5] Vascular comorbidity may be present in over 30% patients with AD and over 50% patients with VaD may exhibit pathology associated with AD, suggesting a 3.4—73% overlap between AD and VaD.[6] Hence, the diagnosis of dementia is not only difficult but also challenging as in many elderly patients both the entities may coexist in combination with other neurodegenerative diseases (often termed as mixed dementia). The original term multi-infarct dementia is now replaced by a newly updated term, vascular cognitive impairment (VCI), which refers to any cognitive impairment caused by or associated with vascular risk factors and ranges from mild cognitive impairment to overt dementia.[7] VaD may be caused by hemorrhagic, ischemic, and hypoxic injury to the brain. It is a group of heterogeneous disorders in which the presence of ischemia/infarction may cause cognitive decline however, the degree of such impairment is directly proportional to the extent of neuronal damage and location of the lesion.[2,8—10] The National Institute of Neurological Disorders and Stroke and Association internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) states that small vessel disease such as microvascular angiopathy (lacunar infarction), periventricular ischemia, and large vessel athero-embolic disease causing territorial infarction are sufficient to result in cognitive impairment and can be included as a criterion of VaD diagnosis.[1,2,11] Several possible pathogenic factors, such as accumulation of advanced glycation end-products and activation of proinflammatory cytokines [interleukin (IL)-1β, tumor necrosis factor (TNF)α, IL-6, and nuclear factor-κB], and experimental studies on animals and cultured neurons have demonstrated that oxidative stress, mitochondrial dysfunction, inflammatory response and accumulation of abnormal amyloid-β have been proposed for the etiopathogenesis of VaD.[4] As per California criteria, diagnosis of VaD requires neuropathological assessment, computed tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS).[2,12—14] Fig. 1 is a systematic diagram illustrating various factors involved in the pathogenesis and risk factors of VaD. In principle, the risk factors for stroke are also the risk factors for dementia. Clinically-evident hypertension has been shown to have significant association with dementia while hyperlipidemia and metabolic syndrome could be predictive of dementia risk.[2,15—17] Transient ischemic attacks also predispose to increase the risk of stroke and 30% of the patients who suffer stroke develop dementia after a period of 6—12 months. Although the exact etiopathogenesis of VaD remains unknown, diabetes mellitus, hormone replacement therapy for postmenopausal women, obesity, improper dietary habits including: food rich in *trans*-saturated fats and lacking omega-3 fatty acids (docohexanoic acid and eicosapentanoic acid) and polyunsaturated fatty acids (linoeic acid, linolinic acid, and arachidonic acid), various environmental neurotoxins; drug addiction; aging; and unhealthy life style including sedentary life style, lack of exercise, physical and mental stress, overmedication, and late night sleep have been proposed in the etiopathogenesis of VaD and can enhance the disease process. Several of these risk factors, apart from genetic, may be prevented by diet manipulation, moderate exercise, and lifestyle modifications. Age of onset of stroke and lack of education have also been associated with higher risk of dementia.[2] Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), an inherited disorder, manifested as syndrome of migraine, mood disorder, recurrent transient ischemic attacks, stroke, and early development of dementia is an independent age-related pathogenesis of AD and VaD; the root cause is primarily microvascular disease. Due to the numerous attributable risk factors detailed studies are needed to understand the exact etiopathogenesis of VaD.

In the present report, we describe systematically recent updates on various *in-vitro* biomarkers from serum and cerebrospinal fluid (CSF) samples and *in-vivo* multimodality neuroimaging biomarkers for the effective clinical management of VaD. It is expected that the information in this review will be of significant interest to medical students, clinicians, and researchers interested in understanding further about this devastating progressive neurodegenerative disorder of unknown etiopathogenesis.

Classification of biomarkers in VaD

Accurate classification of dementia would significantly impact its treatment. Currently, there is no approved drug for the treatment of VaD, so it is exceedingly important to reduce the risk factors and provide adequate treatment

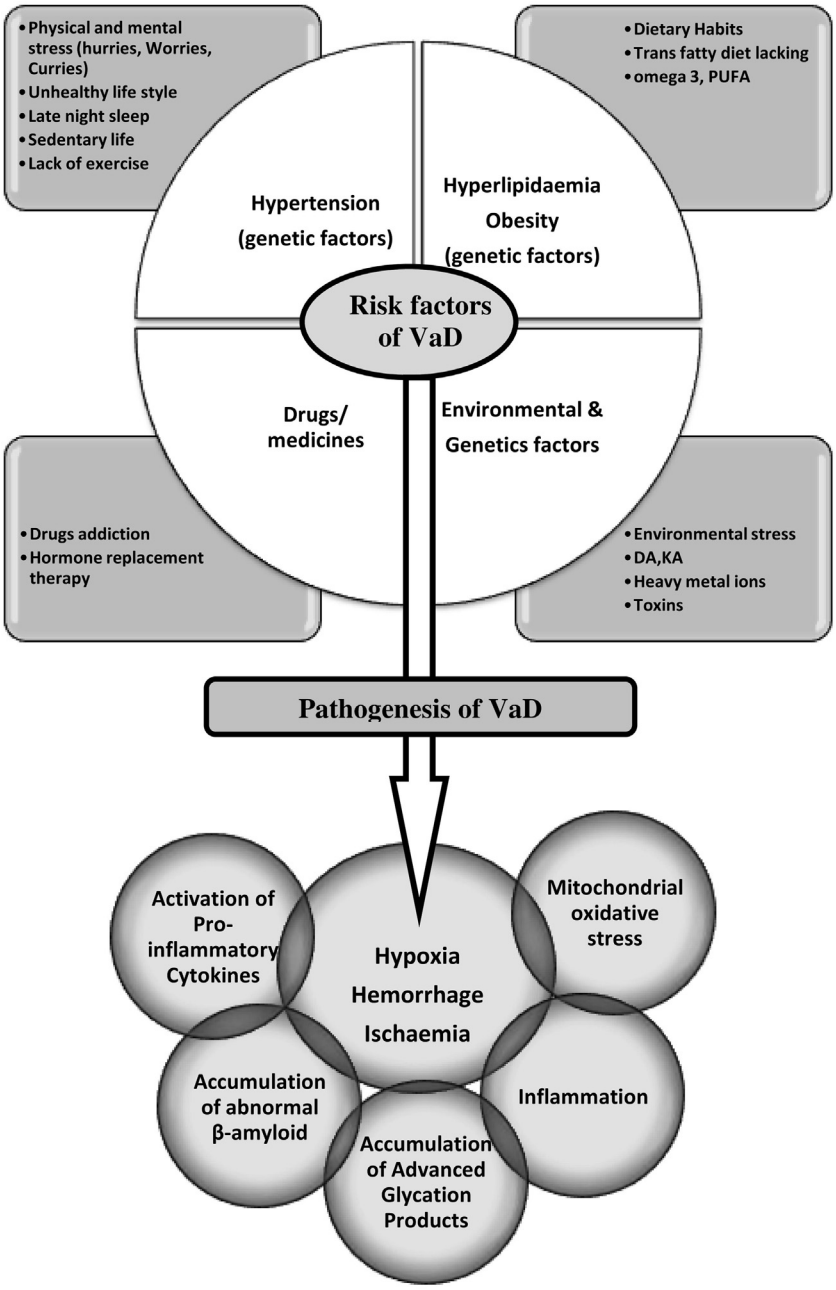

Figure 1 Risk factors and pathogenesis of vascular dementia (VaD). A pictorial diagram illuatrating various risk factors including hypertension, hyperlipidemia, obesity, drugs of abuse, overmedication, hormone replacement therapy, and environmental factors could lead to the pathogenesis of VaD. Activation of proinflammatory cytokines, abnormal accumulation of amyloid-β, accumulation of advanced glycation products, inflammation, and mitochondrial oxidative stress are caused by hypoxia, hemorrhage, and ischemia, resulting in the pathogenesis of VaD.

with proper vascular agents for preventing and/or prolonging the onset of dementia. The major risk factors, hypertension, and hyperlipidemia, can be controlled only with conventional treatment to reduce the risk of VaD. Therefore there is a dire need to classify and determine the specific etiology of dementia based on the biomarkers analyses. These biomarkers must be easily measurable to facilitate early and accurate diagnosis. In this regard, the surrogate biomarkers of VaD, based on functional neuroimaging, and CSF- and blood-based analysis have gained importance as these are noninvasive or minimally invasive, easy to perform as compared to pathological analysis on postmortem brain samples. Moreover, *premortem* analysis of these biomarkers would enhance the diagnostic capability of VaD.[18] The most significant VaD biomarkers that could be used for the early clinical diagnosis, prognosis, and treatment of VaD are presented in Fig. 2. These can be broadly classified as: clinical biomarkers (neurobehavioral assessment); pathological biomarkers (identifying cellular/histological changes); biochemical biomarkers (serum,

plasma, CSF biomarkers); neuroimaging biomarkers, which include functional multimodality fusion imaging with CT, MRI/MRS, PET, and single photon emission CT (SPECT) to derive structural as well as functional information simultaneously regarding the diseases process, genetic biomarkers (identifying genes involved in cerbrovascular disease); and omics and microRNA biomarkers (identifying subcellular components of VaD). Simonsen et al recently described laboratory methods for collection, detection of proteins in CSF and plasma along with purification of candidate biomarkers (chromatographic and electrophoresis technique) and protein profiling using multiplex enzyme-linked immunosorbent assay, surface enhanced laser desorption/ionization time of flight mass spectrometry, and peptide mass fingerprinting.[5] Various statistical methods to calculate probability, sensitivity, and specificity have been described to determine the utility of these biomarkers. Although several biomarkers have been correlated with underlying pathological processes at the cellular and molecular level; the biochemical biomarkers have been directly correlated with clinical and imaging findings in dementia. However, their pathological correlation is yet to be established.[19,20] Humpel reported that the detection of biomarkers in CSF can only support the clinical diagnosis of VaD.[21] Recent studies on biomarkers have emphasized on inflammation, hemostasis, oxidative stress, hypoxia—ischemia, accumulation of biochemical substances, complex proteins, and other metabolites in the hypertensive—atheromatous disease and hyperlipidemia in tissue and CSF.[4,22]

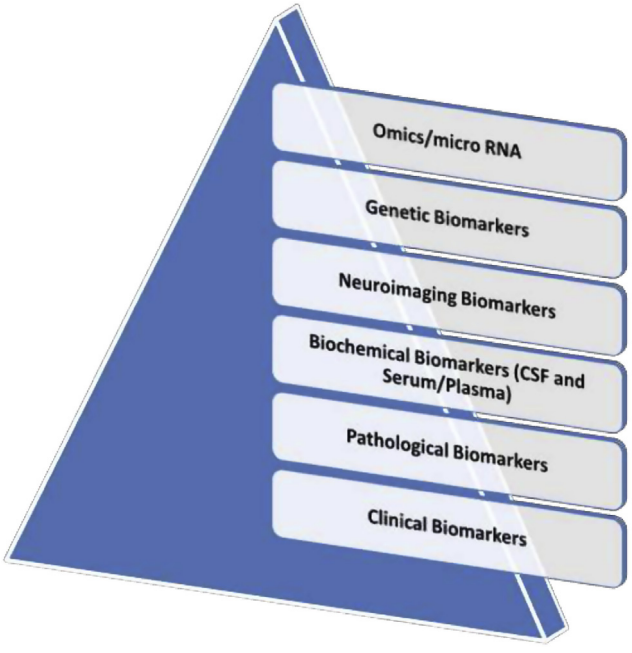

Figure 2 Classification of vascular dementia (VaD) biomarkers. A diagram demonstrating various biomarkers in VaD such as: omics/microRNA, genetic biomarkers, neuroimaging biomarkers, cerebrospinal fluid (CSF) and serum biochemical biomarkers, pathological biomarkers, and clinical biomarkers, which can be used for the differential diagnosis, prognosis, and effective treatment of VaD.

CSF biomarkers of VaD

Although biomarkers can be measured in various body fluids such as saliva, blood, and urine and tissue, CSF has been studied extensively because it drains the ventricular system of the brain and concentration of various metabolites may directly reflect various pathological processes in the brain providing a lead to develop sensitive and specific biomarker in CSF to differentially diagnose various etiological types of dementia.[21] CSF biomarkers in dementia have been reported, especially in patients with AD on a larger scale and have shown promise as sensitive diagnostic tool; however, very few studies are yet available and the candidate CSF biomarkers studied so far show conflicting results and lack specificity due to the heterogenous nature of VaD. As Table 1 summarizes, the protein biomarkers that can be used qualitatively and quantitatively; although they are not specific to VaD when used in combination, they can increase the diagnostic certainty of VaD. CSF serum albumin ratio, CSF index, and CSF total protein are biomarkers having high diagnostic value as these can identify structural and functional integrity of the blood—brain barrier and microvascular damage. An increased albumin level and increased index in VaD patients are well established, but they are nonspecific and may not distinguish VaD from AD.[23,24] Sulfatide a maker for demyelination, is used to identify the extent of demyelination in the white matter and it is found to be elevated in VaD as reported by Tullberg et al[25] and Fredman et al.[26] The cytoskeletal organelle, neurofilament is estimated to identify axonal degeneration and the extent of white matter damage, and is found to be increased in CSF of patients with VaD but not with AD pathology, reflecting the axonal damage that is characteristic of VaD.[24,27] Furthermore, the matrix metalloproteases (MMPs) in the CSF, can be estimated to identify changes in the extracellular matrix associated with vascular diseases with inflammation.[28] MMPs attack the myelin and are regarded as biomarkers of demyelination. Various studies including autopsy studies have shown that MMPs are increased in patients with VaD.[29,30] Certain CSF biomarkers were used earlier for the evaluation of diagnostic utility in AD. Conflicting results in multiple studies have been reported and their potential utility lies in differentiating VaD from AD and the other neurodegenerative diseases. However, many studies show significant overlap between levels in VaD and AD.[5,6] Serum to CSF folate ratio can be used to differentiate VaD from AD. This ratio is significantly reduced in VaD. The reduced folate ratio has been found to be a characteristic of VaD.[5,31] In addition AD is characterized by amyloid (A)β plaque deposition irrespective of its etiopathogenesis. Amyloid-β peptide (1—42) (Aβ-42) is formed after amyloid-β is cleaved from amyloid precursor protein by secretases. A significant reduction of Aβ-42 in patients with AD as well as VaD suggests a significant overlap making it difficult to distinguish AD from VaD.[5,6] Aβ-42, total tau and phosphorylated tau (p-tau) have been extensively studied in AD and there are several reports on their utility in diagnosis and prognosis of AD. Increased levels of tau and decreased levels of Aβ-42 have been detected in AD as well as VaD but more specifically in AD. Hence a combined analysis of these CSF biomarkers has

Table 1 Cerebrospinal fluid (CSF) biomarkers with high diagnostic utility: (Biomarker levels in CSF are raised in vascular dementia, VaD).

Biomarkers	Diagnostic utility
CSF:serum albumin ratio, CSF total protein	To identify blood–brain barrier damage to the small intravascular vessels
Sulfatide	To identify demyelination of white matter
Neurofilament	To identify axonal degeneration (marker of white matter damage)
Matrix metalloproteases	To identify changes in the extracellular matrix associated with cardiovascular disease (i.e. vascular disease with inflammation)
Serum to CSF Folate ratio	Low ratio in VaD
Increased total tau, p-tau, decreased amyloid β42	May differentiate VaD from Alzheimer's disease and other NDD (Neurodegenerative Diseases)

been recommended for the differential diagnosis of VaD.[20,32–35] The protein biomarkers as mentioned in Fig. 3 represent various physiological processes such as protein degradation (ubiquitin), protease inhibition (cystatin C and α1 anti-chymotrypsin), inflammation (C3a, C4a) are known to be associated with neurodegenerative diseases including all forms of dementia. However, their diagnostic utility is enhanced when used in combination with folate ratio, Aβ-42, total tau, or p-tau levels. Simonsen et al conducted the first study to establish the status of these candidate biomarkers in VaD patients.[5] These markers lack specificity and need to be validated and investigated in large prospective multicentric trials. A biomarker of neuronal death, heart fatty acid binding protein is elevated in CSF from patients with various neurodegenerative diseases. Although heart fatty acid binding protein can be detected in early VaD and AD, it lacks specificity.[36]

Serum and plasma biomarkers of VaD

Apart from CSF, certain biomarkers have been identified in the serum and plasma from the blood samples of patients with VaD, AD, and other neurodegenerative diseases. C-reactive protein is an inflammatory biomarker and its levels are elevated in VaD. Hyperhomocysteinemia is a well-established vascular risk factor and increased level of serum homocysteine proves a causal relationship with vascular lesions and thereby VaD.[37,38] Elevated levels of serum homocysteine were also seen in AD patients and are considered to contribute to vascular pathogenesis of AD. Recently, many studies have shown that elevated serum homocysteine is associated with hippocampal and cortical atrophy in patients with VaD.[39] Although deficiency of vitamin B_{12} and folate causes hyperhomocysteinemia, the supplementation of these vitamins failed to produce any improvement in patients with dementia; hence role of homocysteine remains controversial.[40] Elevated lipoprotein-a is considered an independent genetic risk factor for VaD but not in AD, which helps in understanding the pathogenesis of atherogenic processes in VaD.[41,42] Dehydroepiandrosterone (DHEA), a neurosteroid, and its metabolite, DHEA sulfate (DHEA-S) have neuroprotective effects and their levels in the central nervous system are raised in neurodegenerative diseases however, the reason for their altered levels in blood as a cause or as an effect remains uncertain.[43–45] Serum level of DHEA-S is unaltered as reported by a few studies in patients with VaD. A detailed study is needed to determine the exact clinical significance of these and other biomarkers in the differential diagnosis of VaD, as several factors may influence their levels in blood. Furthermore, these biomarker studies should be correlated and confirmed with imaging and histopathological evidence to authenticate VaD diagnosis. Similarly oxidative stressors such as malondialdehyde (MDA), thyroid stimulating hormone (TSH), calcium, and magnesium have been found to be nonspecifically elevated in patients with dementia, suggesting vascular etiopathogenesis in dementing illnesses.[46] The receptor for advanced glycation end products (RAGE) is a cell-bound receptor of the immunoglobulin superfamily that may be activated by proinflammatory ligands including advanced glycol-oxidation end products and amyloid-β peptide. Clinical studies have shown that higher plasma levels of RAGE are associated with reduced risk of coronary artery disease, hypertension, metabolic syndrome, arthritis, and AD.[47] Similarly, atherosclerotic cerebrovascular disease is a significant cause of VaD. So, the protective nature of this biomarker requires further validation. Geroldi et al recently demonstrated that only RAGE and β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE-1) proved to be predictor of cognitive impairment after stroke but there was no association with neprilysin or apolipoprotein E (ApoE).[48] It needs to be determined whether these biomarkers help in distinguishing VaD from vascular cognitive impairment after acute ischemic stroke. Increased levels of thrombin, D-dimer, and thrombin fragment 1+2, and biomarkers of endothelial dysfunction (von Willebrand factor and plasminogen activator inhibitor) are associated with cerebrovascular thrombosis and thereby VaD as illustrated in Fig. 4. Such association may be secondary to chronic inflammation. These mechanisms may underlie prothrombotic state, cerebral microinfarction, and eventually subcortical small vessel infarction. Most of the cases of dementia have mixed etiopathogenesis, contributing a variable amount of vascular pathology (Neuropathology group of the Medical Research Council Cognitive Function and Ageing Study).[49,50]

Genetic biomarkers of VaD

Identifying new risk factors for ischemic stroke could help improve prevention strategies and identify new therapeutic targets. Genetic risk factors are particularly interesting,

because they can offer a direct clue to the biological pathways involved. Ischemic stroke is a heterogeneous disorder, and must be considered for genetic susceptibility factors. In Western countries, most ischemic strokes can be attributed to large-artery atherosclerosis (atherothrombotic stroke) and small-artery occlusion (lacunar stroke).[51] The genes underlying VCI must be of two exclusive classes: (1) genes that predispose individuals to cerebrovascular disease, and (2) genes that determine tissue responses to cerebrovascular disease (e.g. genes conveying ischemic tolerance or susceptibility, or the ability to recover from ischemic insult).[52] In the first category, genes that confer susceptibility to hypertension and atherosclerosis have been identified with some monogenic forms of disease such as CADASIL caused by mutations in NOTCH 3 gene. From the second category; genes that modify tissue responses to injury have also been identified and at least three sets of genes in the AD pathway, the presenilins, APP, and APOE are known to interact with the VCI disease pathway. The presenilin mutations causing AD have been shown to interact directly with Notch proteins, including Notch 3 (mutations of which cause CADASIL).[53–55] There is direct evidence from both human and animal studies for specific non-AD genes that play a significant role in tissue responses in ischemia. Earlier studies in humans suggest that variants in the genes for platelet glycoprotein and α-fibrinogen affect post stroke outcomes without affecting stroke risk *per se*. Animal studies have suggested glutamate and γ-aminobutyric acid receptors, acid-sensing ion channels, proteases, growth factors and their receptors, and transcription factors as the major molecules involved in influencing brain responses to cerebrovascular injury.[56,57] In addition, chromosome 9p21.3 genotype has been associated with VaD and AD.[58] Linkage and association analyses (including single nucleotide polymorphism EDN1, MHTFR, NOS3, and ApoE 4) and AGTR1, AGTR2 of renal angiotensin system) have shown the association of these genes with pathogenesis of small vessel disease, cardiovascular disease (CVD), and VaD. The genes/molecules described in Fig. 5 have been studied extensively in relation to CVD and attempts are being made to determine predisposition to CVD and VaD.[51,59] Genetic diseases such as sickle cell disease, Fabry disease, and homocysteinuria, and genes involved in inflammation (LTC4S, IL-6), thrombosis (TGB3, factor VIII), lipid metabolism (ApoE, PON 1 PON2, PON3, ApoA5, LPL, LDL), endothelial function and oxidative stress (NOS3, MTHFR) and genes identified through linkage analysis in an Icelandic population (ALOX5, PDE4d) are all candidate biomarkers to establish association with cerebrovascular disease, ischemia-stroke, and VaD.[51] There are limited studies available regarding the genetic biomarkers in VaD (Fig. 6). Hence potential genetic and molecular biomarkers of VaD such as genes responsible for cerebrovascular disease, genes influencing the native tissue response and molecules such as soluble receptors for various metabolites and enzymes identified in VaD, have been proposed as

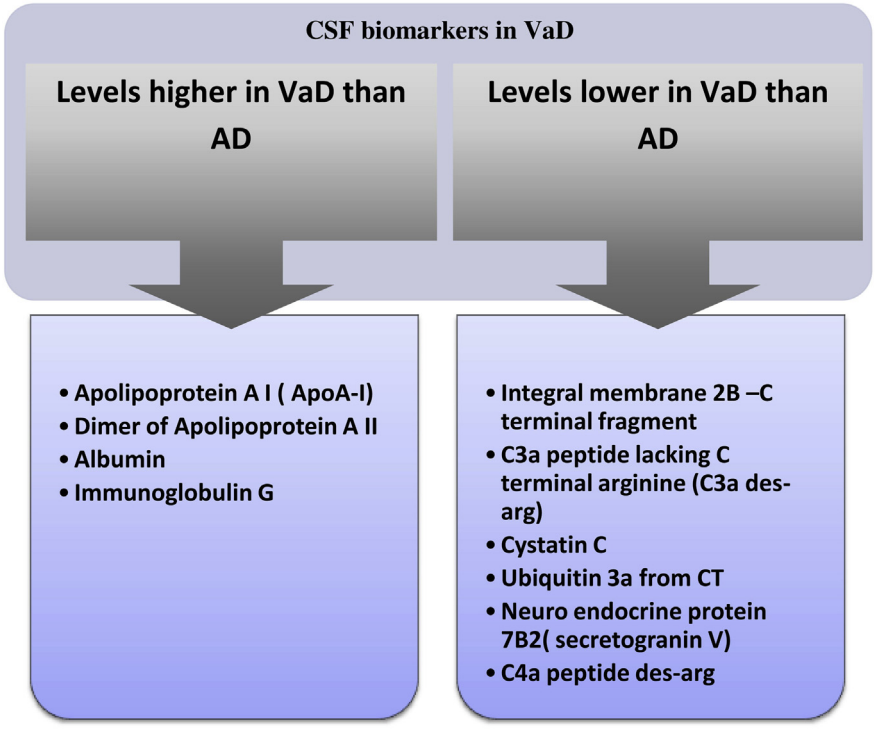

Figure 3 Cerebrospinal fluid (CSF) protein biomarkers mainly used in combination. A pictorial representation of CSF biomarkers that are altered in vascular dementia (VaD) and Alzheimer's disease (AD). These biomarkers possess moderate diagnostic utility. Apolipoprotein-A1, dimers of apolipoprotein-A2, albumin, and immunoglobulin-G have higher levels in the CSF samples of VaD compared to AD patients, whereas integral membrane 2B—C, terminal fragment, C3a peptide lacking c-terminal arginine, cystatin-C, ubiquitin-3a from computed tomography, neuroendocrine protein 7B2 (secretogranin V), and C4a peptide des-Arg levels are lower in VaD as compared to AD patients.

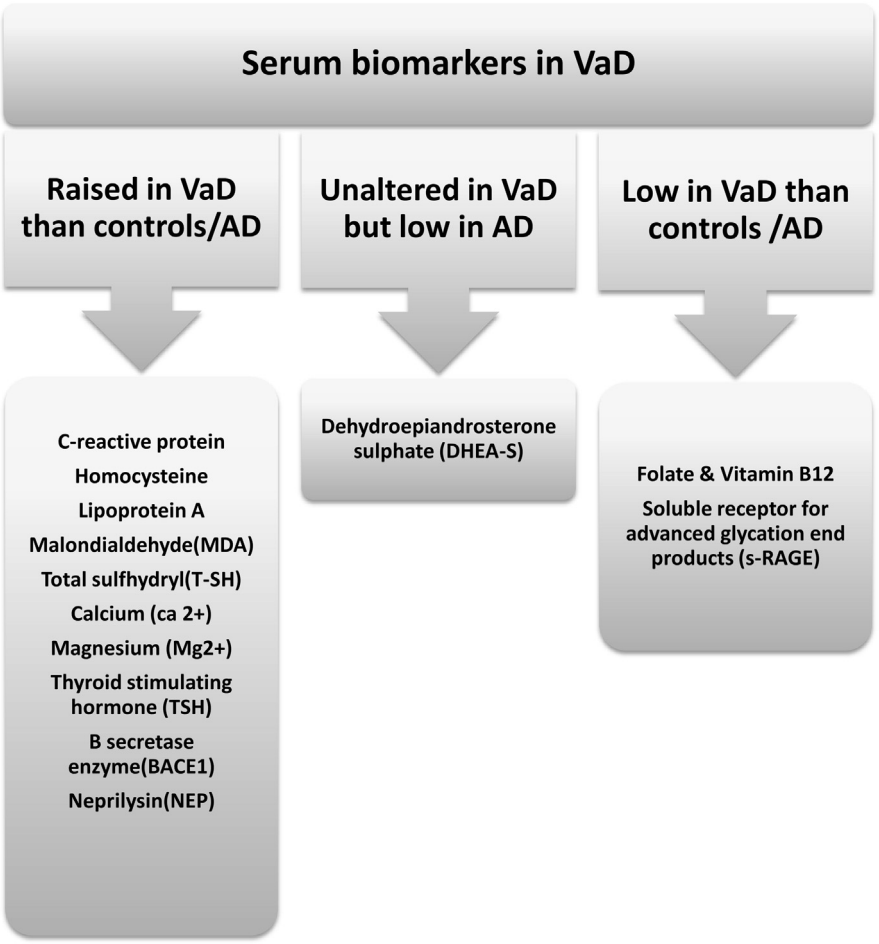

Figure 4 Candidate vascular dementia (VaD) biomarkers in plasma/serum. C-reactive protein, homocysteine, lipoprotein-A, malondialdehyde, total —SH, calcium, magnesium, thyroid stimulating hormone (TSH) β-secratase, neprilysin levels are increased in VaD as compared to normal control and AD patients. DHEA-S levels remain unaltered in VaD but are reduced in AD patients. Folate and vitamin B_{12} and s-RAGE are lowered in VaD as compared to AD patients.

potential biomarkers to correlate with the pathogenesis of VaD. However, further studies are needed to establish their clinical significance.[47,60] The proposed biomarker genes predisposing to cerebrovascular disease are ACE, AGT, eNOS, PON, MTHFR, MEF2A, ALOX5, LTA, APOM, and PDE4D. Certain genes can influence the brain tissue response to VaD such as neurotrophic factors: brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), vascular growth factors, ApoE, MMPs, glutamate, and GABA receptors, adhesion molecules, transcription factors, ion channels, and NOS pathway genes. Genes/loci that are known to alter risk of VaD in a community are PDE4D, ALOX5AP, LTA4H, 9p21, 4q25, ApoE 4 in CADASIL, specific pathway genes, APP, PPAR-γ, LPL, and LIPC.[51,59]

MicroRNA biomarkers in VaD

Various types of microRNAs (miRs) are impaired due to abnormal adipogenesis in obesity to influence the genetic predisposition of VaD. A further study is required to determine their exact significance in the clinical management of VaD. Further studies employing omics biotechnology and miR analysis would provide precise knowledge regarding the exact etiopathogenesis and clinical management of VaD in future. Early mild cognitive impairment syndrome *in vitro* can be estimated by quantitative analysis of brain-enriched cell-free miR in the blood using quantitative real-time polymerase chain reaction. As miRs are important epigenetic regulators of numerous cellular processes including neurodegenerative diseases, specific miRs such as the miR-132 and miR-134 families paired with miR-491-5p and miR-370, respectively, have proven to be the best, detecting mild cognitive impairment of varied etiology and AD. The use of brain-enriched neurites/synapses miR enables detection of early pathologic events occurring in degenerating neurons.[61] Numerous miRs including guardian of endothelial cells, miR 126 (lowered level) and others are found in vascular inflammatory processes, and could serve as biomarkers of early detection of vascular cognitive impairment. Also, therapeutic potential of miRs is a future challenge. The invention of novel modifications of RNA bases and the synthesis of artificial antisense miR or antagomir, may be used as novel therapeutic tools to manipulate miR and control vascular inflammatory diseases.[62] Although free radicals can induce inflammation

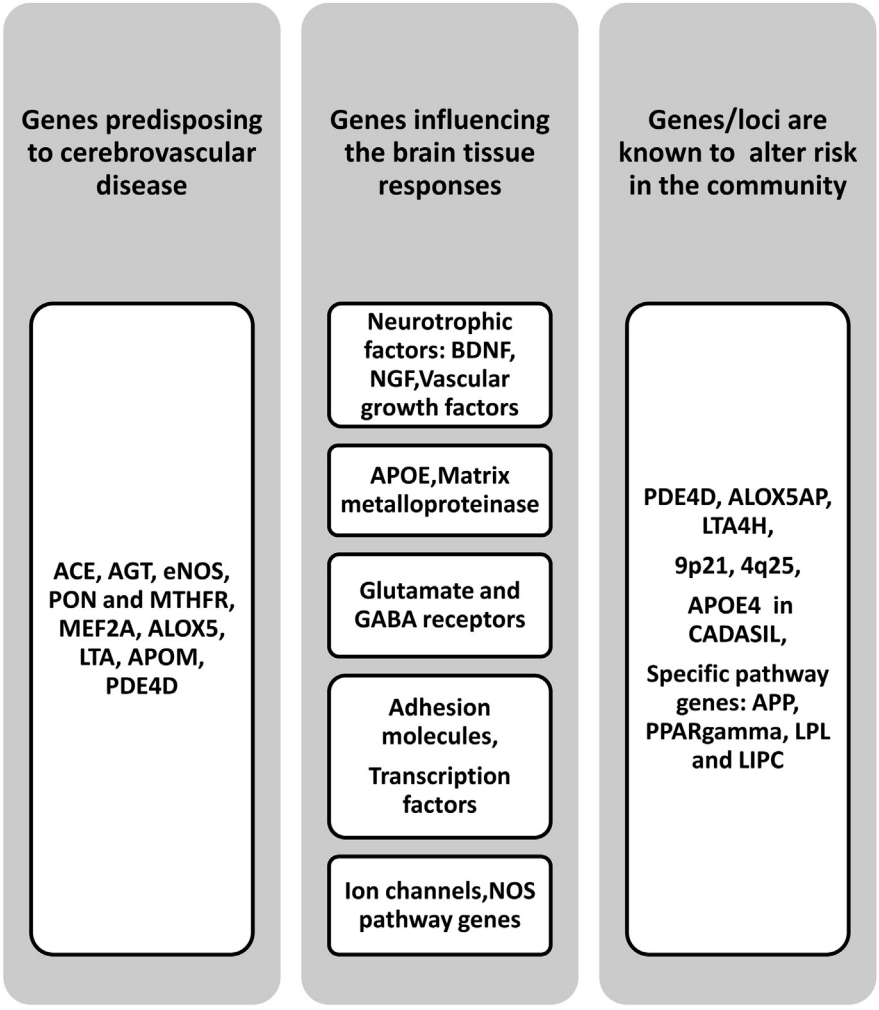

Figure 5 Molecular biomarkers in vascular dementia (VaD). A pictorial diagram illustrating genes such as ACE, AGT, eNOS, PON, MTHFR, MEF2A, ALOX5, LTA, APOM, and PDE4D are the genes predisposing to cerebrovascular disease. Genes which influence the brain tissue responses include neurotropic factors brain-derived growth factor (BDNF), nerve growth factor (NGF), and vascular endothelial-derived growth factor (VEGF), apolipoprotein-E (APOE), and matrix metalloproteinases, glutamate and GABA receptors, adhesion molecules, transcription factors, ion channels, NOS pathways genes. The genes known to alter risk of cerebrovascular diseases in community are: PDE4D, ALOX-5AP, LTA4H, chromosome-9 p21, 4q25, APO-4 in CADASIL, and specific pathway genes such as APP, PPAR-γ, LPL, and LIPC.

by activating redox-sensitive proinflammatory transcription factors, the endothelial dysfunction induced by oxidative stress can release vascular endothelial-derived growth factors (VEGFs) and prostanoids promoting vascular leakage, protein extravasation, and cytokine production. Inflammation enhances oxidative stress by upregulating the expression of reactive oxygen species-producing enzymes and downregulating antioxidant defenses. miRs of these transcription factors can act as potential biomarkers in circulation for VaD. A study published by Ungvari et al, suggests Dicer1 (ribonuclease III) is a key enzyme of the miR machinery, which is responsible for synthesis of mature functional miRs.[63] There is evidence that Dicer1 in endothelial cells may regulate angiogenic processes, a biomarker to be explored as therapeutic target. Role of dysregulation of Dicer1 in age-related impairment of angiogenesis identified a number of miRs that are downregulated in cerebromicrovascular endothelial cells in dementia. Aging results in cerebromicrovascular rarefaction and cerebral angiogenesis is impaired in response to hypoxia or VEGF administration. This plays a prominent role in impairment of regional cerebral blood flow and the occurrence of VCI with age. Because the role of miRNA regulation and function in the aging vascular system is an emerging area, further research is needed to study the contribution of individual miRs or miR families in gene expression that underlie microvascular aging and thereby, VaD.

Biomarkers of cell-based therapy for VaD

There are few studies as yet available on the therapeutic potential of cell-based therapy in VaD. Laboratory studies have shown that transplanted bone marrow stem cells improve neurological diseases of the CNS by generating

neural cells or myelin-producing oligodendroglial cells and enhancing neural plasticity.[64–68] However there are few objective data providing evidence for clinical improvement. Sharma et al administered autologous bone marrow derived mononuclear cells, intrathecally to a 61-year-old woman who was diagnosed with VaD.[69] After follow-up of 2 years she showed clinical improvements as assessed by mini-mental state examination and functional independence measure along with PET/CT neuroimaging exhibiting improved metabolic activity providing evidence of benefits of cell-based therapy and suggestion to investigate various stem cell biomarkers employing omics biotechnology in future studies on VaD.

Recently, significant efforts have been made to explore the basic molecular mechanisms of atherosclerosis (the underlying cause of cerebrovascular and cardiovascular disease), which remains a major cause of morbidity and mortality worldwide. Because of the complex pathophysiology of cardiovascular disease, different research methods have been combined to unravel genetic aspects, molecular pathways, and cellular functions involved in atherogenesis, vascular inflammation, and dyslipidemia to gain a multifaceted picture addressing this complexity. Recent evolution of high-throughput technologies is able to generate data at the DNA, RNA, and protein levels with sophisticated computational technology. These data sets are integrated to enhance information and are being used as regulated networks. Doring et al described genomics, transcriptomics, proteomics, and epigenomics—and systems biology to explore pathomechanisms of vascular inflammation and atherosclerosis.[70] Cerebrolysin, a naturally occurring substance represents a therapeutic strategy for neurological disorders like dementia, stroke, and traumatic brain injury.[71] It is a neuropeptide mimicking the action of neurotrophic factors that enhances neurogenesis, sustaining the brain's self-repair, promotes neural progenitor cell migration, synaptic density rebuilding neuronal cytoarchitecture, restorative processes, decreases the infarct volume and edema formation and promotes functional recovery. Since mitochondrial redox balance is impaired as a consequence of brain regional cerebrovascular insufficiency, antioxidants such as quercetin and isoquercitrin as natural flavonoids help to provide mitochondrial neuroprotection in VaD. Similarly, melatonin reduces free radical generation by enhancing glutathione levels. Neurotrophic factors such as NGF, glial cell-derived neurotrophic factor, and BDNF have already been implicated as targets for treatment of degenerative diseases. These neurotropic factors are generally present in significantly high amounts in the bone marrow-derived mononuclear cells. A recombinant DNA vaccine composed of domains of neurite outgrowth inhibitors. The immunological mechanism inducing effective antibodies against the specific domains and the modulation of mRNA expression regarding neurite outgrowth inhibitors, which help in repair/regeneration of neural and oligodendrocytic damage. Stem cells might be an alternative to brain regeneration. In experimental models of acute ischemic stroke using Q-dot labeled mononuclear cells, we have established that these cells exhibit preferential chemotaxis in the peri-infarcted region and are exponentially eliminated as a function of time. Although the exact molecular mechanism of

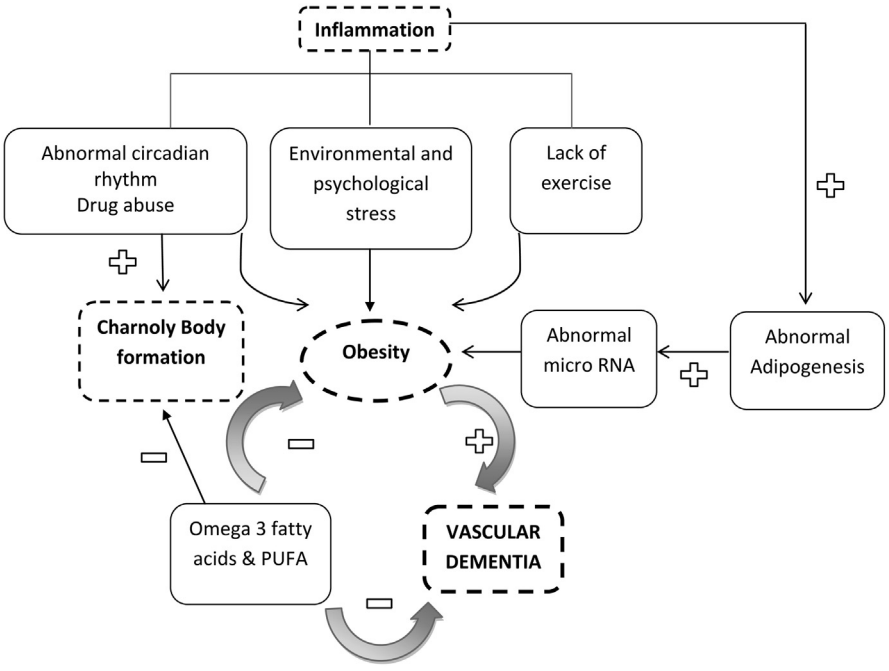

Figure 6 Regulation of vascular dementia (VaD) biomarkers by omega-3 and polyunsaturated fatty acid (PUFA). A diagram demonstrating various risk factors including altered circadian rhythms, drug abuse, environmental and psychological stress, and lack of exercise can induce CB formation, and obesity due to abnormal microRNA and abnormal adipogenesis as a consequence of leptin and orexin gene dysregulation. Omega-3 fatty and PUFA prevent vascular dementia by providing new membrane synthesis and stabilization.

neuroprotection offered by mononuclear cells remains enigmatic, it is assumed that the neuroprotection is provided by autocrine and paracrine mechanism by local release of neuroprotective biomarkers such as insulin-like growth factor, endothelial derived growth factor, von Wilebrand factor, and granulocyte colony stimulating factor, IL-4, and IL-10 as anti-inflammatory cytokines. Naive human chorionic villi and amniotic fluid derived cells release significant amounts of BDNF, as well as VEGF. Nimodipine, as an L-type voltage-dependent Ca^{2+} channel antagonist and an antihypertensive agent, can also reduce ischemic nerve cell death in VaD. Further studies in this direction promise to discover sensitive and specific biomarkers of VaD.

Neuroimaging biomarkers

Neuroimaging has been extensively studied in various types of dementia including VaD. In particular, CT and MRI specific changes have been identified as potential biomarkers demonstrating mechanisms of vascular injury and their effects in the parenchyma, which can be detected in all the stages of VaD.[1] Phase contrast MRI and the analyses of hemodynamics in the brain have also been regarded as potential biomarkers, although their sensitivity and specificity need considerable evaluation. Neuroimaging findings correlate very well with the underlying pathological processes and hence have gained importance in research and clinical investigation on VaD.[1,28] Significant findings on standard and routine neuroimaging techniques that can be utilized as biomarkers in the diagnosis of VaD are summarized in Table 2. It has been proved that T2 weighted MRI sequences alone or in combination with CT can identify leukoaraiosis (white matter lesions), microvascular angiopathy, lacunar infarction, dilation of Virchow—Robin spaces, pulse wave encephalopathy, parameters of cerebral embolic disease, which have been correlated with *postmortem* findings of vascular pathology of dementia.[1] However, there is still a need for the development of imaging parameters having diagnostic utility but also having capacity of determining etiopathogenesis, differentiating VaD from AD and other neurodegenerative diseases. Ligand-specific PET and SPECT will serve as future diagnostic methods when these ligands are developed for different proteins found in VaD e.g. Tau, Aβ 40 and many others. Arterial spin labeling, which measures absolute blood flow though cerebral vessels may offer better results than SPECT in detecting hypoperfused areas. Moreover it is cost effective and avoids use of radioactive substances. Functional MRI can assess neuronal function through blood oxygen level-dependent changes. Although the neurovascular mechanism underlying blood oxygen level-dependent changes is still poorly understood, functional MRI is being used in neurological research. T_2-T_2'-T_2 relaxometry and susceptibility-weighted MRI takes into account not only the magnitude but also the phase and signals for gradient echo MRI sequence. Susceptibility-weighted MRI, which has ability to differentiate calcium, iron and hemorrhagic products, can be a promising biomarker in differentiating the aging brain from VaD.[72]

Pathological biomarkers of VaD

The definitive diagnosis of VaD depends on the histopathological analysis of *postmortem* brain samples or animal models, which not only confirm the specificity of biomarkers but also facilitate classifying the disease process at the cellular and molecular level. The characteristic pathology such as microvascular angiopathy, CADASIL, hypertensive vasculopathy, cerebral amyloid angiopathy (CAA), and atheroembolic or thrombotic diseases have been identified and well-documented.[1,7,73]

Table 2 Imaging biomarkers in vascular dementia (VaD).

Imaging method	Biomarkers—salient features in VaD	Diagnostic utility
Magnetic resonance imaging (T1,T2 weighted and FLAIR images)	Deep white matter hyperintensity	Strongly correlated with small vessel CVD, embolic disease and VaD (Mills)
	Periventricular hyperintensity	Associated with CVD, ischemic disease
	Infarction (lacunar, site specific such as basal ganglia—cystic lesions, number, and size)	Strongly associated with CVD and VaD
	High signals in basal ganglia	Represents atherosclerotic arteries and VaD
	Dilated Virchow—Robin space	Strongly related to VaD on autopsy studies.
	Pulse wave encephalopathy seen as lacunar infarction and white matter hyperintensity	Related to abnormal pulse pressure and Windkessel effect leading to CVD
	Hemorrhage (number, size and location)	Associated with CVD
	Brain atrophy	Estimates age related, site related changes and vascular pathology
Computed tomography scan	Ventricular size, medial temporal atrophy, acute or chronic hemorrhage, hypodensities defining infarction as per size, location	Data not validated. Association with VaD is not yet proven.
Transcaranial Doppler	Spontaneous cerebral emboli	Related to embolic infarct and VaD

CVD = cardiovascular disease.

Various pathological VaD biomarkers are illustrated in Table 3. Theses biomarkers can be divided into six major categories: (1) biomarkers of CADASIL; (2) biomarkers of microvessel angiopathy; (3) biomarkers of hypertensive vasculopathy; (4) biomarkers of cerebral amyloid angiopathy; (5) biomarkers of atherosclerosis or thrombotic disease; and (6) CB formation due to mitochondrial degeneration and eventually apoptosis of the most vulnerable cells in the hippocampal dentate gyrus and CA-3 regions due to cerebrovascular insufficiency in VaD.[14,74–80]

Clinical biomarkers in VaD

Clinical assessment of VaD is based on neurobehavioral biomarkers that are assessed by performing mental status examination (MSE). MSE evaluates the extent of intellectual deterioration and personality change. This is followed by language performance test to acquire high yield results for the clinical assessment of VaD.[81] Among the different types of MSEs, Mini MSE of Folstein, Hachinski ischemic score scale, and Wechsler adult intelligence scale have been found to be most useful. These tests use evaluation of attention span, temporal, and spatial orientation and retentive (declarative) memory. A score < 23 on Mini MSE of Folstein is usually diagnostic of dementia. Further typing is based on identification of risk factors as in VaD.[82,83] Furthermore, the Hachinski ischemic score scale is a simple bedside clinical biomarker and currently used for differentiating types of dementia (primary degenerative, vascular, multi infarct, mixed type). A cut-off score ≤ 4 for dementia of other types and ≥ 7 for VaD has a sensitivity of 89% and a specificity of 89%.[84,85] The Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) Criteria for VaD take into account memory impairment, one of the cognitive disturbances such as aphasia, apraxia, agnosia and laboratory imaging findings in support of vascular etiology.[13] International Classification of Diseases-10 Research Criteria (DCR-10) for VaD is similar to DSM IV criteria with additional evaluation of consciousness, and decline in emotional and social behavior. AD Diagnostic and Treatment Centers Criteria for the Diagnosis of Probable Ischemic VaD takes into consideration memory decline, history of vascular risk factors, neurological signs, and neuroimaging findings, relatively early appearance of gait disturbance and urinary incontinence to favor diagnosis of dementia with probable ischemic etiology. Ischemic scores classified as VD by different diagnostic guidelines set by Hachinski as follows: score indicating VaD ≥ 7; AD Diagnostic and Treatment Centers criteria: 10.3 ± 3.4; DSM-IV criteria: 6.5 ± 4.4; DCR-10 criteria: 7.9 ± 4.0; NINDS-AIREN criteria: 12.5 ± 2.6. Orthostatic circulatory disturbances such as alteration in mean arterial pressure, postural hypotension have been shown to be associated

Table 3 Neuropathological biomarkers.

Category	Biomarkers	Diagnostic utility
Biomarkers of CADASIL (Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy)	CADASIL, CRV, HERNS	Familial small vessel diseases
Biomarkers of microvascular angiopathy	Thickening of small vessel wall, luminal narrowing, degeneration of tunica media, fibrinoid necrosis, inflammation Vasculitides	Assesses microvascular angiopathy Noncerebral amyloid angiopathy associated angiopathy
Biomarkers of hypertensive vasculopathy	Circle of Willis assessment, Presence of aneurysm, stenosis of vessels	Marker for atherosclerosis
Biomarkers of CAA (Cerebral amyloid angiopathy)	Amyloid deposition in vessels on routine H&E staining and Congo red + β amyloid antibody staining	Indicator of CAA
Biomarkers of atherosclerosis or thrombotic disease	1. Presence of ischemia or hemorrhage 2. Presence of infarcts: number, size, location, acute or chronic, cystic, watershed, lacunar (white matter, grey matter, brain stem), laminar necrosis, hippocampal injury, cribriform change. 3. Incomplete ischemic injury 4. Loss of myelin on H&E and special stain such as LFB	Assesses cerebrovascular injury Strong parameters of CVD and VaD, correlated clinically and with imaging findings CVD Leukoencephalopathy.
Charnoly body (CB): A universal pre-apoptotic biomarker of compromised mitochondrial bioenergetics and cell injury	Hippocampal lesions	Need to be validated and distinguished form lesions in Alzheimer's disease (CB as a biomarker of compromised mitochondrial bioenergetics)
Miscellaneous	Mixed-multiple pathology	CVD

CVD = cardiovascular disease; H&E = hematoxylin and eosin; VaD = vascular dementia.

with development of VaD and in some other neurodegenerative diseases.[86–88]

Limitations in biomarker studies

Although biomarkers have been studied extensively, there is no consensus on several issues such as definitions of standard procedures, uniqueness of processing and storage, analysis and interpretation of results and their diagnostic utility. It is important to identify a biomarker that is not only specific but also stable. In routine clinical practice it is desirable to use the biomarkers with stability due to constraints of time and handling of specimens e.g. RNA stabilizers or exclusion of RNA chips, use of anticoagulants may give rise to variable results. Large multicentric trials are necessary to compare diagnostic accuracy of different laboratories all over the world.[21] There are limitations in use of different analytical methods. For instance, enzyme-linked immunosorbent assay, used routinely may differ from Luminex's xMAP technology when CSF and plasma samples are analyzed, which also affects the cut off values hence, standardization of international standard values is required. Validation of biomarkers in body fluids need standardization with universal and valid criteria for clinical diagnosis, defining healthy controls, reproducibility in multiple centers and correlated with standard *postmortem* diagnosis. CSF diagnosis of dementia supports only clinical and not *postmortem* diagnosis. Novel potential biomarkers of VaD such as asymmetrical dimethylarginine, which is a biomarker of endothelial dysfunction, adhesion molecule P-selectin may contribute to vascular processes and thereby dementia need larger studies and validation.[7] Currently CSF isoprostane, a biomarker of oxidative stress, Aβ oligomer, α synuclein, TDP-43, CSF DJ-1, TDP-43 are being investigated in AD. As dementia is defined as mixed etiology, there is a dire need to consider all these biomarkers along with newer biomarkers of vascular injury in determining causal relationship to VaD. Innovative neuroimaging techniques such as diffusion tensor imaging, MRS, functional MRI, amyloid-β PET imaging may provide newer insights in the etiopathogenesis of VaD.[89] Gadolinium diethylene triamine penta-acetic acid also has shown promise in detecting vascular pathology in dementia; however, all these imaging biomarkers need further evaluation through multicentric trials. Dysfunction of autonomic regulation of cerebral blood flow is also associated with VaD where imaging biomarkers may provide diagnostic utility.[7] There has not been any consensus on criteria, definitions, or analysis of VaD in neuropathological assessment. Although numerous gross and microscopic changes have been identified as diagnostic of VaD, there is a need of multidisciplinary team performing large multicenter clinicopathological studies and harmonize the diagnostic approach and validate the biomarkers under investigation; e.g. abandonment of the term lacunae, which is a source of confusion; reducing interobserver variability.[24] Genetics and molecular biology may show a definitive avenue towards diagnosis and behavior of VaD; genome-wide association studies have become technically feasible but are still expensive.

Conclusion

In this communication, we have reviewed the recent literature on the development of VaD biomarkers. A variety of candidate biomarkers identified in CSF and blood by neuroimaging methods, neuropathological examination, and genetic analysis have shown promise in their utility as biomarker for etiological diagnosis and behavior of vascular cognitive impairment and VaD however, lack of specificity, lack of criteria to identify and define the components of VaD prompts further large scale studies and evaluation of these biomarkers and need to develop novel biomarkers. The recent discovery of CB formation as a preapoptotic biomarker of oxidative stress and compromised mitochondrial bioenergetics may serve as a novel biomarker of VaD. In addition, biomarkers of oxidative and nitrative stress in serum and CSF samples can be detected for the early diagnosis, treatment and prognosis of VaD. A further study in this direction will go a long way in the clinical management of VaD.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

The authors express their sincere thanks to Kallol Guha, President, Saint James School of Medicine, Bonaire for his moral support and encouragement.

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