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DIFFERENCES IN VASCULAR RISK FACTOR FREQUENCY AMONG PATIENTS WITH CEREBRAL SMALL VESSEL DISEASE WITH AND WITHOUT CONSEQUENTIAL ISCHEMIC STROKE

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RAZLIKE U UČESTALOSTI VASKULARNIH FAKTORA RIZIKA KOD BOLESNIKA SA BOLEŠĆU MALIH KRVNIH SUDOVA MOZGA SA I BEZ POSLEDIČNOG ISHEMIJSKOG MOŽDANOG UDARA

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Dedicated to all those trying to find their path in life

“Do or do not, there is no try”

– Master Yoda, “Star Wars: Episode V - The Empire Strikes Back”

Abstract

DIFFERENCES IN VASCULAR RISK FACTOR FREQUENCY AMONG PATIENTS WITH CEREBRAL SMALL VESSEL DISEASE WITH AND WITHOUT CONSEQUENTIAL ISCHEMIC STROKE

Introduction: Cerebral small vessel disease (CSVD), most commonly arteriolosclerosis, and large vessel disease (large artery acute ischemic stroke – LAAIS) share risk factors including hypertension, diabetes, dyslipidemia, smoking, atherosclerosis, kidney dysfunction, inflammation, and blood-brain barrier dysfunction.

Aims: The aim was to assess risk factor prevalence in CSVD, find the differences between patients with and without LAAIS, and determine their influence on the occurrence of LAAIS by calculating the odds ratio (OR) and relative risk (RR).

Methods: This study included 241 CSVD patients. Data on demographics (age, sex), patients' medical histories (history of LAAIS, its severity, and localization), carotid ultrasonography, and risk factors (dyslipidemia, serum lipids, diabetes mellitus, hypertension, smoking, atherosclerotic disease, kidney dysfunction, liver enzymes, vitamin B12, folic acid, coagulation, complete blood count, cerebrospinal fluid (CSF) analysis) were collected and neutrophil to lymphocyte ratio (NLR) and CSF to serum protein ratio calculated. The data on disease imaging biomarkers was collected, and the disease burden was calculated.

Results: LAAIS patients had greater carotid stenosis, burden score, NLR, CSF to serum protein ratio, and more atherosclerotic disease, but lower total and HDL cholesterol ($p<0.05$). Higher odds of LAAIS were found with carotid plaques (OR 2.38), disease imaging burden (OR 2.67), and atherosclerotic disease (OR 3.22). The highest risk was found with severe carotid atherosclerosis (RR 2.57), atherosclerotic disease (RR 1.54), and NLR (RR 1.49).

Conclusion: Atherosclerosis markers, imaging burden, and inflammation can be used to identify CSVD patients who would benefit from additional follow-up and early management to prevent LAAIS.

Keywords: cerebral small vessel disease, vascular risk factors, atherosclerosis, carotid stenosis, carotid ultrasound, transcranial ultrasound, stroke, magnetic resonance imaging

Scientific field: Medicine

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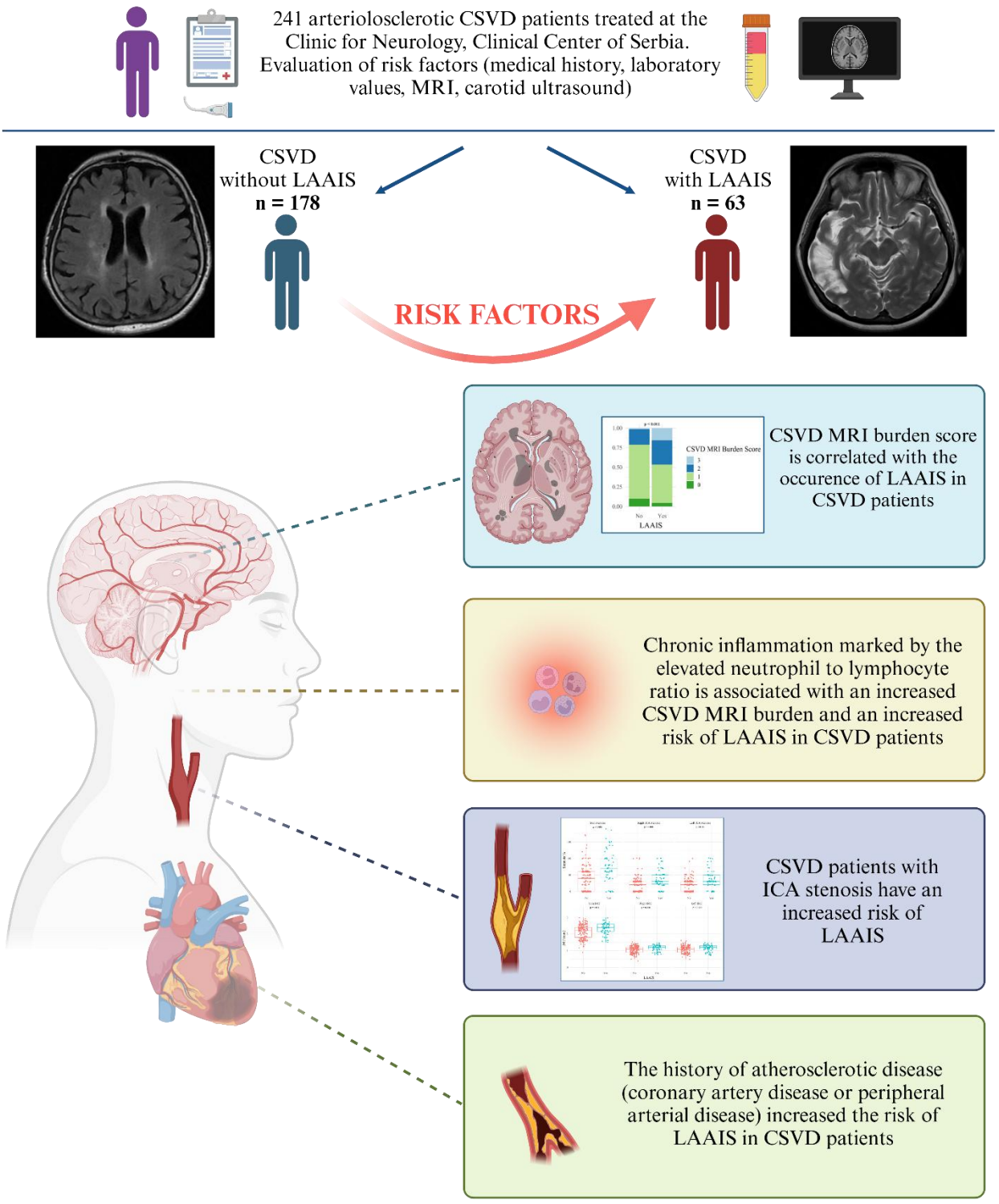
UDK Number:

Graphical Abstract



Sažetak

RAZLIKE U UČESTALOSTI VASKULARNIH FAKTORA RIZIKA KOD BOLESNIKA SA BOLEŠĆU MALIH KRVNIH SUDOVA MOZGA SA I BEZ POSLEDIČNOG ISHEMIJSKOG MOŽDANOG UDARA

Uvod: Bolest malih krvnih sudova mozga (BMKS) i bolest velikih krvnih sudova (ishemijski moždani udar – IMU) dele faktore rizika uključujući hipertenziju, dijabetes, dislipidemiju, pušenje, aterosklerozu, bubrežnu bolest, zapaljenje, i disfunkciju krvno-moždane barijere.

Cilj: Cilj rada je utvrđivanje učestalosti faktora rizika kod BMKS bolesnika, poređenje razlika između onih sa i bez IMU, i utvrđivanje njihovih uticaja na pojavu IMU kroz izračunavanje odnosa šansi (OŠ) i relativnog rizika (RR).

Metode: Ova studija je uključila 241 BMKS bolesnika. Podaci o demografiji (godine, pol), medicinskoj istoriji (IMU, njegova težina i lokalizacija), karotidnoj ultrasonografiji, i faktorima rizika (dislipidemija, vrednosti lipida, dijabetes, vrednosti glukoze, hipertenzija, pušenje, aterosklerotska bolest, bubrežna bolest, enzimi jetre, vitamin B12, folna kiselina, koagulacija, krvna slika, analiza cerebrospinalne tečnosti (CST)) su prikupljeni, a odnos neutrofila i limfocita (ONL) i odnos proteina CST i seruma su izračunati. Podaci o radiološkim biomarkerima su prikupljeni i opterećenje bolešću izračunato.

Rezultati: Pacijenti sa IMU su imali više vrednosti karotidne stenoze, opterećenja bolešću, ONL, odnos proteina CST i seruma, i više aterosklerotske bolesti, ali niži ukupni i HDL holesterol ($p<0.05$). Veće šanse za IMU su otkrivene sa karotidnim plakovima (OŠ 2,38), višim opterećenjem bolešću (OŠ 2,67), i aterosklerotskom bolesti (OŠ 3,22). Najviši rizici su nađeni sa visokostepenom karotidnom aterosklerozom (RR 2,57), aterosklerotskom bolesti (RR 1,54), i ONL (RR 1,49).

Zaključak: Markeri ateroskleroze, opterećenje bolešću, i zapaljenje mogu biti korisni za identifikaciju BMSK bolesnika koji bi imali koristi od učestalijeg praćenja i rane terapije radi prevencije IMU.

Ključne reči: bolest malih krvnih sudova mozga, vaskularni faktori rizika, ateroskleroza, karotidna stenoza, ultrazvuk karotidne arterije, transkranijalni ultrazvuk, moždani udar, magnetna rezonanca

Naučna oblast: Medicina

Uža naučna oblast: Neurologija

UDK Broj:

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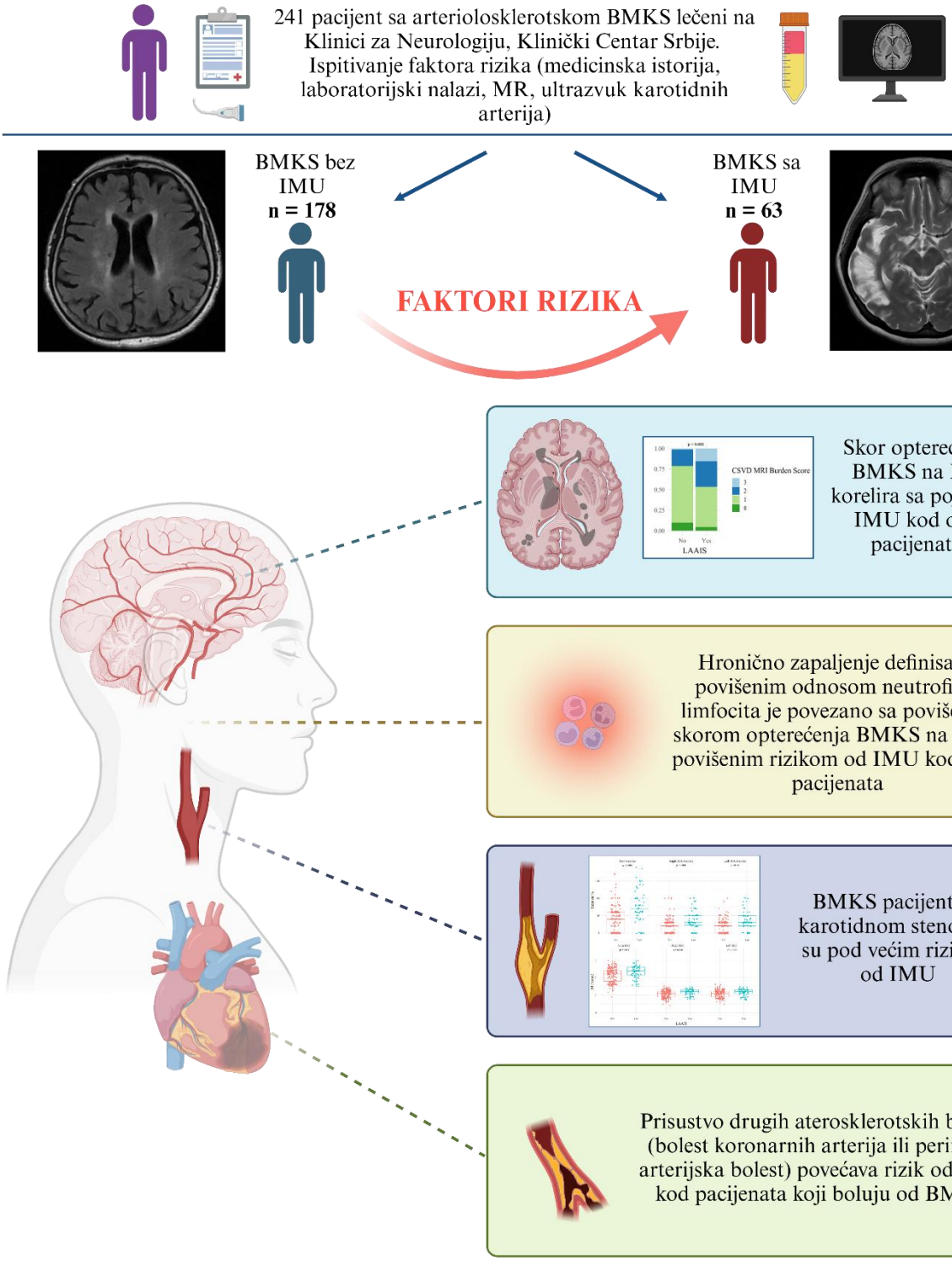

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1. INTRODUCTION

1.1. Definition and basic anatomy of cerebral small vessels

Cerebral small vessels include vessels of the brain around 50 to 400 μm in size, meaning arterioles, capillaries, and venules. Arterioles of the brain are small arterial vessels derived from two main sources: the arteries of the circle of Willis (including anterior, middle, and posterior cerebral arteries) and the leptomeningeal vasoganglion (1).

The brain in its entirety is supplied by a pair of internal carotid arteries (ICAs) and a pair of vertebral arteries (VAs) (Figure 1). The right and left common carotid arteries give rise to the corresponding ICA. Each ICA then enters the skull and bifurcates, giving rise to the anterior cerebral artery (ACA) and middle cerebral artery (MCA). The two VAs enter the skull through the foramen magnum and fuse near the pontomedullary junction, thus forming the basilar artery (BA). BA later gives rise to two posterior cerebral arteries (PCAs). At the base of the skull, the arteries of the anterior circulation (ACA and ICA) and arteries of the posterior circulation (PCA) anastomose via communicating arteries and form the circle of Willis. As these arteries branch out to supply the brain, they give rise to the penetrating arterioles and later parenchymal arterioles that supply the subcortical parenchyma (2).

Leptomeningeal vasoganglion is a network of leptomeningeal arteries that covers the pial surface of the brain and allows the anastomosis between the large arteries of the circle of Willis. Branches of the leptomeningeal arteries penetrate the glia to reach the cortex (2). The gray matter contains most of the brain arterioles, about 8 times more than the white matter (3). Histologically, arteries of the brain are muscular arteries made up of 3 layers. Tunica intima is the inner layer made up of endothelial cells arranged in a layer and internal elastic lamina. Tunica media is the middle layer containing the smooth muscle cells. Unlike other arteries, cerebral arteries don't contain an external elastic lamina. Lastly, the tunica adventitia is the outer layer consisting mostly of collagen and fibroblasts. This layer is in direct contact with other cells of the brain, including pericytes and astrocytes, or with perivascular nerves, like in the case of small leptomeningeal arteries. As arteries get smaller, the histology changes, so they contain fewer and fewer smooth muscle cells. In the end, penetrating arterioles have just a single layer of smooth muscle cells in their media (2).

Arterioles of the brain drain into the capillaries. It is important to note that capillaries contain about 50% of the total brain blood volume. Histologically, these vessels are made up of a layer of endothelial cells connected by tight junctions (forming the blood-brain barrier – BBB), pericytes, astrocytes, and basal lamina. BBB functions as a semi-permeable membrane that creates a controlled brain microenvironment by regulating the passage of molecules between the blood and brain tissue. Even though the BBB is formed at the level of endothelial cells, there is a complex interaction between all components of the capillaries, known as the neurovascular unit, which has an impact on the function of the BBB (2–4).

The final element of the small brain vessels involves the venules. Venules vastly outnumber the arterioles, as each arteriole is surrounded by eight venules. As with other veins, they have thinner vessel walls and larger lumen than their corresponding arteries. Venules drain the deoxygenated blood from the brain parenchyma centrifugally towards the cortex and venous sinuses (3).

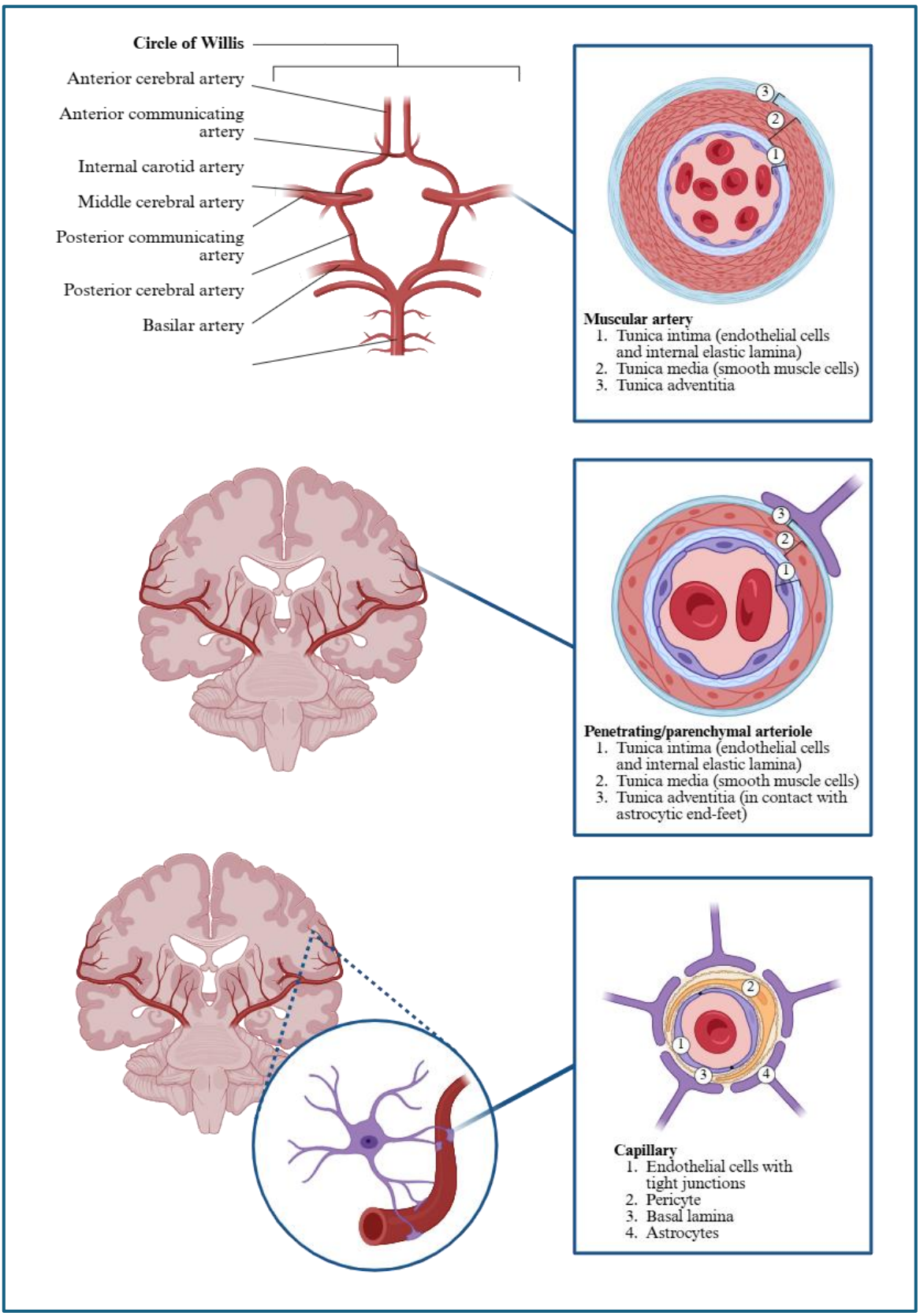

Figure 1. Anatomic and histological characteristics of cerebral vasculature. Created in BioRender.com

1.2. Definition and epidemiology of cerebral small vessel disease

As the name implies, cerebral small vessel disease (CSVD) is a condition that affects small vessels of the brain supplying the white matter and the deep gray matter, including arterioles, capillaries, and venules. CSVD is actually an umbrella term used to describe chronic and progressive pathologic conditions of different etiologies that affect these vessels (5). CSVD is defined by the presence of CSVD-related brain lesions on magnetic resonance imaging (MRI), so-called CSVD imaging markers. These include lacunar strokes (LS), lacunes, white matter hyperintensities (WMH), cerebral microbleeds, enlarged perivascular spaces (EPVS), and brain atrophy (5,6).

Prevalence of CSVD increases with age, with an estimated 5% prevalence in individuals around the age of 50 increasing to almost 100% in those older than 90 (7). Some studies also show the increasing prevalence of CSVD among the aging population of 60 years and older, while noting that only 8% of these individuals are free of CSVD-related brain lesions (8). There is also an increase in disease severity with age, shown by the increasing presence of multiple CSVD imaging markers, which goes from 1.9% in those aged 60 to 46.2% in individuals over the age of 75 (9). Even though the data on the presence of CSVD in younger patients is lacking, some studies demonstrate the increasing prevalence of CSVD in young patients as well. Fan and associates evaluated the prevalence of CSVD in patients ages 18 – 49 with first-ever stroke and found that the prevalence of CSVD-related brain lesions increased with age from 10% in the group of 26 to 29-year-old patients, none of which had WMH, to 33% in those of 46-49 years of age. Out of them, 14% had only LS, 10% only WMH, and 9% had both lesions (10). The prevalence of each CSVD imaging marker varies significantly among different studies, possibly due to the influence of other covariates such as hypertension and the subtype of CSVD. Most authors agree that LS and WMH are the most common findings, while cerebral microbleeds are the least common one. Additionally, it is evident that the prevalence of each CSVD imaging marker increases with age (8,9,11–14). It is suggested that CSVD is more prevalent in the Asian population, possibly due to lifestyle and risk factors, however, these results are difficult to recreate (9,14). When it comes to sex, most authors note that there are no sex differences, however, some have found that women have more CSVD-related brain lesions (8–10).

1.2.1. Etiology of cerebral small vessel disease

All the etiologies that make CSVD can be grouped into 6 major types. Type 1 includes arteriolosclerosis related to age and vascular risk factors (5). Type 2 involves both sporadic and hereditary cerebral amyloid angiopathy (CAA). This type is characterized by the deposition and accumulation of amyloid β in the walls of small cerebral vessels, causing microaneurysms, blood extravasation, and possibly occlusion of the lumen (1,5,15). These small blood extravasations are seen as microbleeds on MRI, which are the most common finding with this type of CSVD (15,16). CAA is a part of Alzheimer's disease (1,5,15). Type 3 involves inherited or genetic CSVD, not including CAA. Some important causes that fall into this category include Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) and Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CARASIL) (5). CADASIL is caused by a mutation in the NOTCH3 gene. Pathological changes occur in the tunica media, where granular osmiophilic material accumulates, leading to the thickening of the vessel wall (15,17). CARASIL is caused by HTR1 gene mutations. In this type, there is smooth muscle cell degeneration, wall thickening, and narrowing of the vessel lumen (15,18). Type 4 CSVD encompasses inflammatory and immunologically mediated CSVD (5). In this type, there is immune cell infiltration and inflammation, causing vasculitis (15). It is associated with EPVS on MRI (16). Type 5 includes venous collagenosis, which involves noninflammatory accumulation of collagen in the venous vessel wall in the periventricular white matter. This type is associated with WMH. The final type of CSVD is type 6, which encompasses other small vessel diseases that are not categorized in the first 5 types. Some examples include cranial radiation and microvessel degeneration in Alzheimer's disease, not related to amyloid accumulation (5,15).

1.3. Pathophysiology of arteriolosclerotic cerebral small vessel disease

The current understanding of CSVD pathophysiology comes primarily from the work of C. Miller Fisher in the 1960s. Fisher performed multiple autopsy studies, examining features of CSVD, mostly LS and lacunes, attributing these lesions to lipohyalinosis and atheromatous occlusions involving fat-filled macrophages. He correlated these lesions to hypertension, as almost all patients in these studies had hypertension (19–22). When computed tomography (CT) imaging was introduced, the research focus switched to imaging, while pathological study efforts nearly ceased (16,23). In recent years, new studies have emerged, focusing on the pathological mechanism and histological changes in CSVD (24).

Arteriolosclerotic CSVD is the most common type of CSVD. It is histologically characterized by loss of vascular smooth muscle cells and elastin fragmentation in the media. Additionally, there is a proliferation of fibroblasts, hyalinization, deposition of collagen, thickening of the vessel wall, and lumen narrowing. Another important feature of this type is microangiopathy, which is pathologically linked to atherosclerosis. Due to these changes, the vessels become elongated and tortuous. As the vessel wall expands, it causes the formation of microaneurysms (1,5,24,25).

Ultimately, changes to vessel physiology lead to functional changes. The BBB becomes impaired, leading to increased permeability of the vessel wall, accumulation of inflammatory cells like lymphocytes and macrophages, and subsequent osmotic demyelination (25). Additionally, arteriolosclerosis causes stiffening of the arteries, impairing vasodilation and cerebral autoregulation (26). Once autoregulation becomes impaired, the vessels lose their ability to adapt to the oxygen needs of the brain parenchyma, and the flow decreases, leading to chronic hypoperfusion of the brain. Occlusion of the small arteries leads to lacunar strokes. On the other hand, severe stenosis of multiple vessels in the white matter leads to ischemia and WMH (1).

1.3.1. “Traditional” vascular risk factors

Modifiable risk factors for CSVD include hypertension, diabetes mellitus (DM), dyslipidemia, smoking, and lack of exercise (1,5,27,28). As mentioned earlier, hypertension has been identified as a risk factor for lacunar strokes by Fisher’s early studies (23). The prevalence of hypertension in these patients is so common that type 1 arteriolosclerotic CSVD is often referred to as hypertensive CSVD (1,5). Some studies linked hypertension with other imaging features of CSVD like WMH and EPVS, noting that WMH severity increases with higher blood pressure and long-standing hypertension, and that individuals with poorly controlled hypertension had more severe WMH (29–32). In fact, hypertension is a common finding among studies on CSVD (33–35). Even with this seemingly clear correlation, some authors find that vascular risk factors, including hypertension, could explain the variance in large vessel atherosclerosis, but not the variance of WMH in the same patients, leading them to conclude that WMH could have a non-vascular origin (36).

Both type 1 and type 2 DM have been linked to CSVD and its imaging features, including microbleeds, WMH, and LS. This connection is clear, as DM is known to cause changes in the microvasculature in the entire body (37–39). Interestingly, one study had unexpected results showing that glycemic control in patients with type 1 DM had no influence on the occurrence of CSVD after 20 years of chronic hyperglycemia (40).

Dyslipidemia is a well-known risk factor for atherosclerotic changes in the brain vasculature. Recent studies focus on finding independent lipid metabolism-related markers of CSVD. For example, Woong Nam et al. identified the atherogenic index of plasma, calculated as a logarithm of the triglyceride to high-density lipoprotein cholesterol (HDL-C) levels, as a potential marker of CSVD, more precisely WMH and LS (41). Yu et al. evaluated how low-density lipoprotein cholesterol (LDL-C) subtypes influence the risk of CSVD. The authors developed a prediction model that included LDL-C3 and LDL-C4 that could help identify high-risk CSVD patients, even in cases of

normal LDL-C levels (42). When discussing vascular risk factors, it is important to mention the effect of metabolic syndrome. Most studies agree that metabolic syndrome is related to CSVD. One study, using the UK Biobank database including around 37000 individuals, showed that patients with metabolic syndrome have larger WMH volume, less brain volume, and poorer cognition (43). Other authors found that metabolic syndrome severity was associated with increased WMH volume and the presence of lacunes, both of which are considered atherosclerotic lesions, but not with microbleeds or EPVS (44). Even when comparing patients with CSVD, those with metabolic syndrome have greater impairment in cognition and worsening of depression (45).

Smoking is also considered a risk factor for CSVD (1,16,28). Smoking is known to induce oxidative stress, trigger the recruitment of leukocytes, and cause matrix metalloproteinase upregulation, thereby creating a pro-atherosclerotic environment. Smoking specifically affects cerebral circulation by causing vasodilatation of small cerebral blood vessels, affecting autoregulation, and increasing BBB permeability (possibly by affecting tight junctions), which in turn affects brain homeostasis (46). Clinical studies have shown a decreased cerebral blood flow in smokers, particularly in the anterior circulation (47). As for CSVD markers specifically, smoking has been correlated with WMH and LS, and to a lesser extent with microbleeds and EPVS (13,48,49). Smoking is also shown to cause CSVD progression, increase CSVD MRI burden, and speed up cognitive decline in these patients (48,50).

Lack of exercise hasn't been thoroughly researched, but some studies show that physical activity in older adults is linked to lower WMH load and less brain atrophy (51,52). Additionally, Roig-Coll et al. measured the effect of physical activity on white matter integrity on MRI, showing a positive correlation and indicating the importance of lifestyle modifications such as increased physical activity on brain health (53).

A major non-modifiable risk factor for CSVD is age. As previously discussed, age is strongly associated with the prevalence of CSVD and disease burden on MRI in both older and younger individuals (7–10). The estimated prevalence of CSVD in individuals around the age of 50 is 5%, while in those who are older than 90, CSVD prevalence is almost 100% (7). Some authors even found a large prevalence of CSVD imaging markers in a cohort of individuals without any signs of CSVD (54). The same trend is present in the younger population. In one study, CSVD prevalence increased with age from 10% in the group of 26 to 29-year-old patients, to 33% in those of 46-49 years of age (10).

Sex is another non-modifiable factor well-researched in CSVD. Unlike age, sex is not clearly correlated with CSVD, and there are many conflicting findings. While some studies attribute female sex as a risk factor for CSVD progression and WMH, others associate male sex with CSVD progression and the occurrence of microbleeds. It is important to note that the definition of disease progression in CSVD is not clear. Some use cognitive decline as a marker of progression, while others focus on neuroimaging findings and the CSVD disease burden on MRI (8,48,50,55). Currently, sex is not considered a risk factor for CSVD, possibly due to conflicting results across studies (5,27,56).

1.3.2. "Non-traditional" risk factors

An obvious choice among risk factors specific to CSVD would be BBB dysfunction, as it is a prominent part of CSVD pathophysiology. Measuring the BBB dysfunction is possible using the cerebrospinal fluid (CSF) to serum albumin ratio. Albumin is produced in the liver and can reach the central nervous system only via the BBB. If the BBB is intact, the values of albumin in the CSF are in normal ranges. However, if there is a BBB dysfunction, albumin levels in CSF increase. The CSF to serum albumin ratio has been extensively researched in dementias and, to some extent, in CSVD, showing association with WMH and the CSVD MRI burden (57–59).

Systemic inflammation has also been proposed as a risk factor for CSVD. Blood neutrophil to lymphocyte ratio (NLR) is a marker of systemic inflammation used across multiple medical disciplines. NLR helps assess the balance between the innate immune response, characterized by neutrophils, and the adaptive immune response, characterized by lymphocytes. The NLR has been shown to be a reliable marker and is associated with aging, coronary artery disease (CAD), stroke, diabetes, and cancers of solid organs. Elevated NLR is also found in the elderly who are at risk of cognitive impairment. In CSVD, elevated NLR has been associated with disease progression, MRI burden, and cognitive impairment (60–62).

Kidney dysfunction is one of the less commonly reported risk factors for CSVD. Kidney dysfunction doesn't actually lead to CSVD, but kidneys and brain share some similarities in vasculature, making them prone to similar pathologies. Therefore, kidney function can be a window into the brain vasculature. Both organs have small perforating arterioles that are able to maintain perfusion through autoregulation. Blood flow is relatively constant in both organs, and both possess a kind of blood barrier allowing them to maintain homeostasis. Markers of CSVD have been correlated with chronic kidney disease (CKD). For example, WMH correlate with a decreased estimated glomerular filtration rate (eGFR). These lesions are also more prevalent in patients with CKD (63,64).

Another organ that is surprisingly related to the brain is the liver. As non-alcoholic fatty liver disease (NAFLD) is becoming more common in the population, it raises the question of whether it has an influence on other organs. NAFLD and CSVD share metabolic risk factors and are both influenced by inflammation, which is proatherogenic in the brain (65). Some studies consider NAFLD and liver fibrosis to be related to brain aging, and to specifically contribute to WMH severity (66,67). Additionally, liver steatosis, fibrosis, and gamma-glutamyl transferase (GGT) levels have been correlated with decreased cerebral blood flow (68).

Hyperhomocysteinemia is considered a risk factor for atherosclerosis, vascular disease, and stroke, all three being components of CSVD. CSVD patients have higher homocysteine levels than those without CSVD (69). Homocysteine levels have also been associated with MRI CSVD burden, most significantly with LS and atrophy (70). Some authors even found that CSVD might act as a mediator between elevated homocysteine and cognitive impairment (71). The neurotoxic effect of homocysteine is well-researched but not completely established. Some theories consider homocysteine to deplete B12 stores from the glial cells, which is why they cannot support neuronal survival. Another possibility is that homocysteine leads to the production of free radicals and apoptosis. Homocysteine might also act on glutamate and N-methyl-D-aspartate (NMDA) receptors, leading to neuronal cell death (72).

Vitamin B12 and folic acid have an established role in brain development and function, and are associated with cognitive health (73,74). Furthermore, vitamin B12 and folic acid are able to lower homocysteine levels, indicating a role for their supplementation in hyperhomocysteinemia. Even though some authors identified a correlation between white matter lesions and B12, follow-up studies assessing the effect of vitamin B12 supplementation on lesion progression over 2 years didn't show a significant reduction (75,76).

Besides vitamins, thyroid hormones have a key role in brain development and functioning. Even after stroke, thyroid hormones regulate the reorganization of the brain (77). In CSVD, increased levels of thyroid-stimulating hormone (TSH) and increased free thyroxine (fT4) have been associated with neuroimaging markers of the disease (78). Additionally, Teng et al. described an association between subclinical hypothyroidism and cognitive impairment in CSVD patients, while Guo et al. found that elevated serum TSH correlated with post-stroke depression after an acute LS (77,79).

Lastly, obstructive sleep apnea is somewhat of a controversial risk factor for CSVD. Even though obstructive sleep apnea is a known risk factor for stroke, studies fail to show a clear correlation with CSVD. Some authors have shown this correlation in patients with transient ischemic attack (TIA) or acute LS, indicating the importance of obstructive sleep apnea as a risk factor for CSVD (80). However, when investigating asymptomatic individuals, sleep apnea doesn't seem to be related to CSVD (81,82).

1.3.3. Association with atherosclerosis

Atherosclerosis is a major component of arteriolosclerotic CSVD. Fisher, in his research, identified pathological markers of atherosclerosis in cerebral small vessels as microatheroma with fat-filled macrophages (5,23). Some authors suggest that stenosis of the penetrating arterioles in LS comes from atheroma in the parent artery, linking large and small vessel atherosclerosis (83). This link has been investigated by multiple studies, with some showing that atherosclerosis in cerebral arteries, but also in other vascular beds like the aorta or coronary arteries, correlated with CSVD severity and progression (54,84,85). As atherosclerosis and CSVD share common risk factors, the association can be assumed (1,5). Most authors link these changes with long-standing hypertension in CSVD patients (29–32). Even though this is the currently accepted mechanism of involvement, some authors challenge this notion. One systematic review included 24 studies on WMH and cerebral blood flow and found that there is a lot of heterogeneity in the included studies, but that cerebral blood flow is negatively related to WMH severity, suggesting that hypoperfusion of the brain is actually the consequence of WMH, not the cause (86).

1.4. Clinical presentation of arteriolosclerotic cerebral small vessel disease

As CSVD is used to describe different etiologies affecting the same blood vessels (cerebral arterioles, capillaries, and venules), the presentation of this condition can vary (1). CSVD is commonly an asymptomatic disease, and it may remain asymptomatic for years (27). Some studies suggest that up to 20% of elderly individuals have some findings related to CSVD (7). CSVD might present acutely, as symptomatic LS, which causes around 25% of all ischemic strokes (6). LS are small strokes up to 20mm in size that usually occur in deep gray matter or subcortical white matter (87). The Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification is used to classify all ischemic strokes into 5 subtypes: large artery atherosclerosis, cardioembolism, small artery occlusion, other known cause, and unknown cause (Figure 2). According to TOAST, LS are classified as type 3, small artery occlusion (88). Most commonly, LS are purely motor, presenting with motor paresis or even paralysis. However, they can be pure sensory strokes that present with unilateral loss of sensation, without any motor symptoms; mixed sensorimotor strokes; ataxic hemiparesis that presents with unilateral weakness and ataxia; or dysarthria-clumsy hand syndrome, in which case patients present with unilateral facial weakness, dysarthria, dysphagia, and hand weakness on the same side (27,87). Fisher was the first to identify these lacunar syndromes in his autopsy studies and correlate them to a specific cerebral territory. Therefore, LS in the internal capsule, corona radiata, or pons lead to pure motor stroke, while LS in the thalamus cause sensory stroke. If LS are found in the thalamocapsular region, they can cause sensorimotor stroke, but those in the pons, midbrain, internal capsule, or parietal white matter might lead to ataxic hemiparesis. The fifth type, dysarthria-clumsy hand syndrome, is caused by LS in the internal capsule and pons (7,22,27). Since subcortical structures are involved, patients with symptomatic LS usually don't have cortical signs like neglect, hemianopsia, or aphasia. Additionally, as these strokes are smaller than other ischemic strokes, they are less likely to cause cerebral edema or post-stroke epilepsy (87). Lacunar strokes are usually minor compared to other ischemic strokes, with a National Institutes of Health Stroke Scale (NIHSS) of about 6 (89). Besides ischemic lesions, CSVD can cause hemorrhagic ones as well. However, intraparenchymal hemorrhage is not a common feature of arteriolosclerotic CSVD. If hemorrhage is

discovered on imaging, there is a higher chance that the patient has another type of CSVD. CAA is particularly known to cause hemorrhagic lesions like microbleeds and intraparenchymal hemorrhage (87).

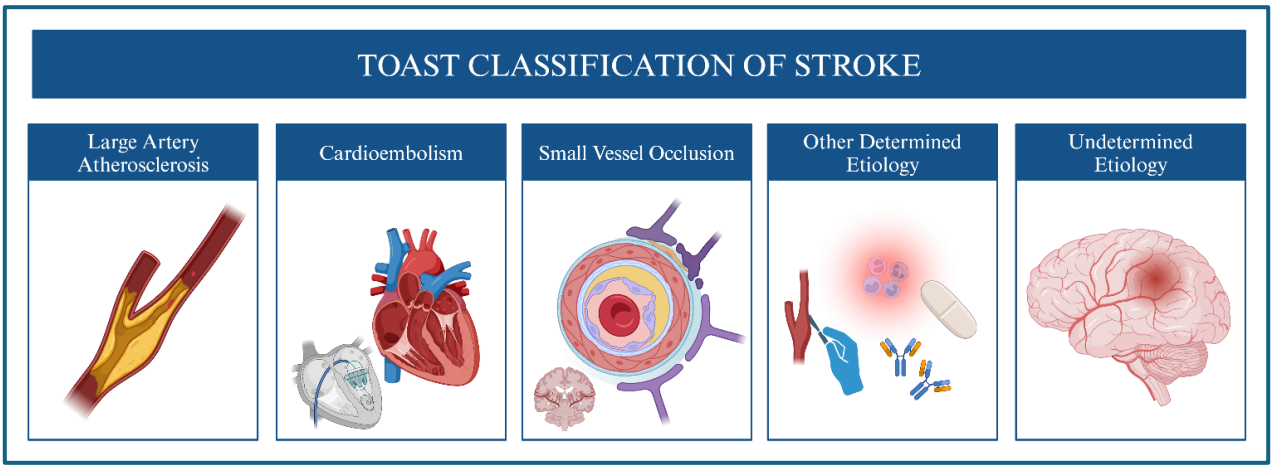

Figure 2. TOAST classification of ischemic stroke. Abbreviations: TOAST - (87) Trial of Org 10172 in acute stroke treatment. Created in Biorender.com

Even though CSVD is not always acute, asymptomatic CSVD over time leads to cerebral ischemia, causing global brain damage. With chronic ischemia, there is a loss of connectivity in the brain, leading to chronic disorders (87). Chronic CSVD is associated with cognitive impairment, depressive symptoms, or gait disturbances (5,27). In fact, CSVD is an important cause of vascular dementia, contributing to around 45% of all cases (90). Cognitive decline in CSVD is usually stepwise and gradual, with slow progression. Cognitive impairment involves executive dysfunction, memory decline, delayed recall, reduced speed of processing information, and reduced verbal fluency. However, episodic memory is relatively preserved (1,87,91). Another chronic manifestation of CSVD involves psychiatric and behavioral alterations. CSVD patients might experience apathy, depression, pseudobulbar affect, irritability, agitation, anxiety, and sleep disturbances. Urinary problems, like incontinence and nocturia, are also common in CSVD (1,7,27). The final chronic consequence of CSVD is gait dysfunction. Gait disturbance in CSVD is characterized by low velocity and short stride, resembling Parkinsonian gait. The two can be differentiated as CSVD gait has a sudden onset and appears later in life (1,7). Gait dysfunction in CSVD is correlated with WMH severity and localization (5,7). However, some authors suggest the involvement of microbleeds, showing that patients with more microbleeds have a shorter stride and lower velocity (92).

1.5. Diagnosis of arteriolosclerotic cerebral small vessel disease

CSVD can be suspected based on the patient's symptoms, but the diagnosis is confirmed with neuroimaging, most commonly MRI. In the case of asymptomatic CSVD, the diagnosis is incidental when imaging is done for another reason (7).

1.5.1. Radiographic criteria

Signs of CSVD on imaging are defined based on the Standards for Reporting Vascular Changes on Neuroimaging (STRIVE) criteria and include LS, lacunes, WMH, EPVS, microbleeds, and atrophy (Figure 3) (6,87). LS are defined as recent small subcortical infarcts. They are considered to be a result of ischemia caused by occlusion of one penetrating artery that occurred a few weeks

prior to imaging. On MRI, LS are defined as oval or round lesions up to 20mm in size, occurring in the white matter, basal ganglia, or the brainstem. LS are seen as hyperintense lesions on diffusion-weighted imaging (DWI), fluid-attenuated inversion recovery (FLAIR), and T2-weighted imaging, while on T1-weighted imaging, they are hypointense. DWI is the most sensitive method for their detection. After the acute phase, LS undergo morphological changes like volume reduction (1,6). Most LS convert into lacunes over time, but it is not clear in what percentage, as studies report the rate of this conversion to range anywhere from 27 to 97% (93–96). Lacunes presumed of vascular origin are defined as round or ovoid lesions 3 to 15mm in size. The definition of size is based on their pathology. Lesions less than 3 mm in size are more probably EPVS. The upper limit of 15mm differs from the upper limit of LS, which is 20mm. That is because LS undergo volume reduction before becoming lacunes, making them smaller. On DWI, lacunes appear hypointense but might be isointense. On T1-weighted imaging, they are hypointense, but hyperintense on T2-weighted MRI. Lacunes are best seen on the FLAIR sequence, where they have CSF-like intensity (hypointense) with a hyperintense rim, which is not always present (6,87).

WMH occur from diffuse hypoperfusion due to reduced cerebral blood flow, resulting in BBB breakdown, glial activation, and demyelination. They appear in the white matter as this area is most vulnerable to hypoxia due to a lack of anastomosis between cerebral arteries. This is known as the watershed effect (1,5). In literature, the term WMH is used interchangeably with leukoencephalopathy, leukoaraiosis, and white matter disease or lesions. According to STRIVE criteria, WMH are confluent areas, isointense on DWI, but isointense or hypointense on T1-weighted imaging. On T2-weighted imaging and FLAIR, WMH appear as hyperintense lesions (6). Periventricular WMH can additionally be graded on the modified Fazekas scale. Grade 1 is defined as a thin lining surrounding the lateral ventricles. Grade 2 is more prominent, appearing as a smooth halo around ventricles, while Grade 3 is seen as hyperintense lesions around lateral ventricles, infiltrating into the deep white matter (97).

EPVS are another important imaging marker of CSVD. Physiologic perivascular spaces, also known as Virchow-Robin spaces, are found around small penetrating cerebral blood vessels that extend from the leptomeninges. These spaces are filled with interstitial fluid (1,6,87). They can become enlarged due to inflammation (15,16). On imaging, EPVS can appear linear if the imaging plane is parallel to the vessel, or round if the imaging plane catches the cross-section of the vessel, with a diameter of less than 3mm. They usually have CSF-like intensity, meaning isointense on DWI, hypointense on FLAIR and T1-weighted imaging, and hyperintense on T2-weighted imaging (6).

Microbleeds are small lesions resulting from the rupture of cerebral small blood vessels, usually found in the cortico-subcortical junction, deep gray, or white matter (1,87). On MRI, microbleeds appear as round or ovoid lesions 2 to 5mm in size but can range up to 10mm (6). They are void of signal on all sequences due to hemosiderin deposits (1). Microbleeds can be specifically imaged on T2-weighted gradient-recalled echo (GRE) MRI, where they are visualized as well-defined hypointense lesions (6). Brain atrophy is another imaging feature of CSVD. It occurs due to cortical thinning and neuronal loss. According to STRIVE criteria, brain atrophy in CSVD patients is defined as a decreased brain volume not related to focal injury like ischemic stroke or trauma, noticed by ventricular and sulcal enlargement (6).

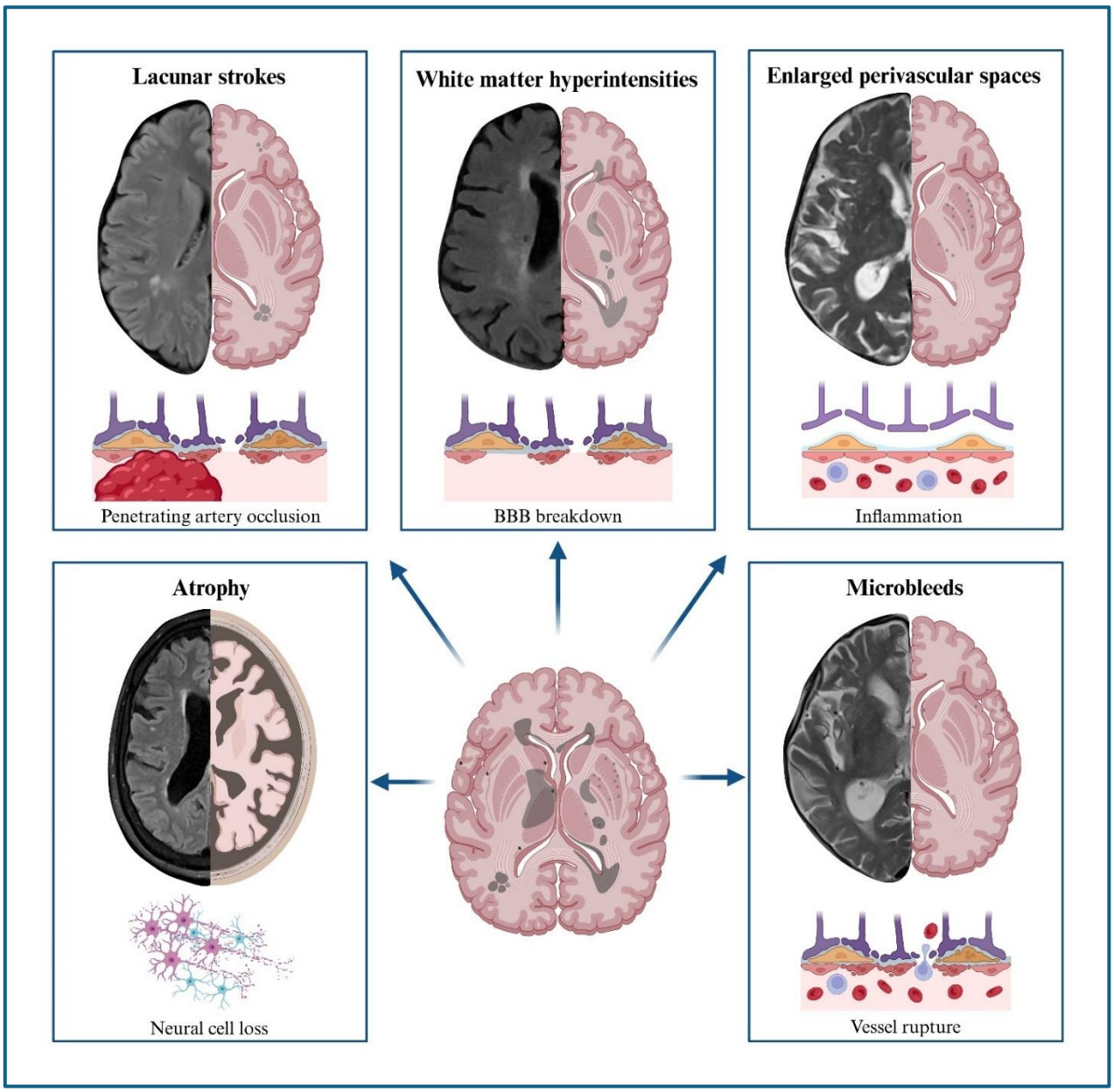

Figure 3. CSVD imaging markers on MRI and their pathological mechanisms. Abbreviations: BBB – blood-brain barrier. Created in Biorender.com

Recently, a scoring system of MRI imaging findings in CSVD has been proposed to measure the lesion burden on the brain (Figure 4). This scoring system includes 4 out of 5 imaging markers of CSVD: LS, WMH, EPVS, and microbleeds. Atrophy was not included, probably due to its high prevalence in the aging population and strong correlation with other conditions. According to this scoring system, asymptomatic LS are awarded a point. WMH of Fazekas score 2 or 3 are also awarded a point. The presence of any deep cerebral microbleeds is awarded a point, and the presence of more than 10 EPVS in the basal ganglia is awarded a point. Total points can range from 0 (e.g., in the case of patients with only symptomatic LS) to 4 (98). A lot of research efforts have been put into this scoring system, correlating some major findings in CSVD and biomarkers with score severity (33,54,59,70,80).

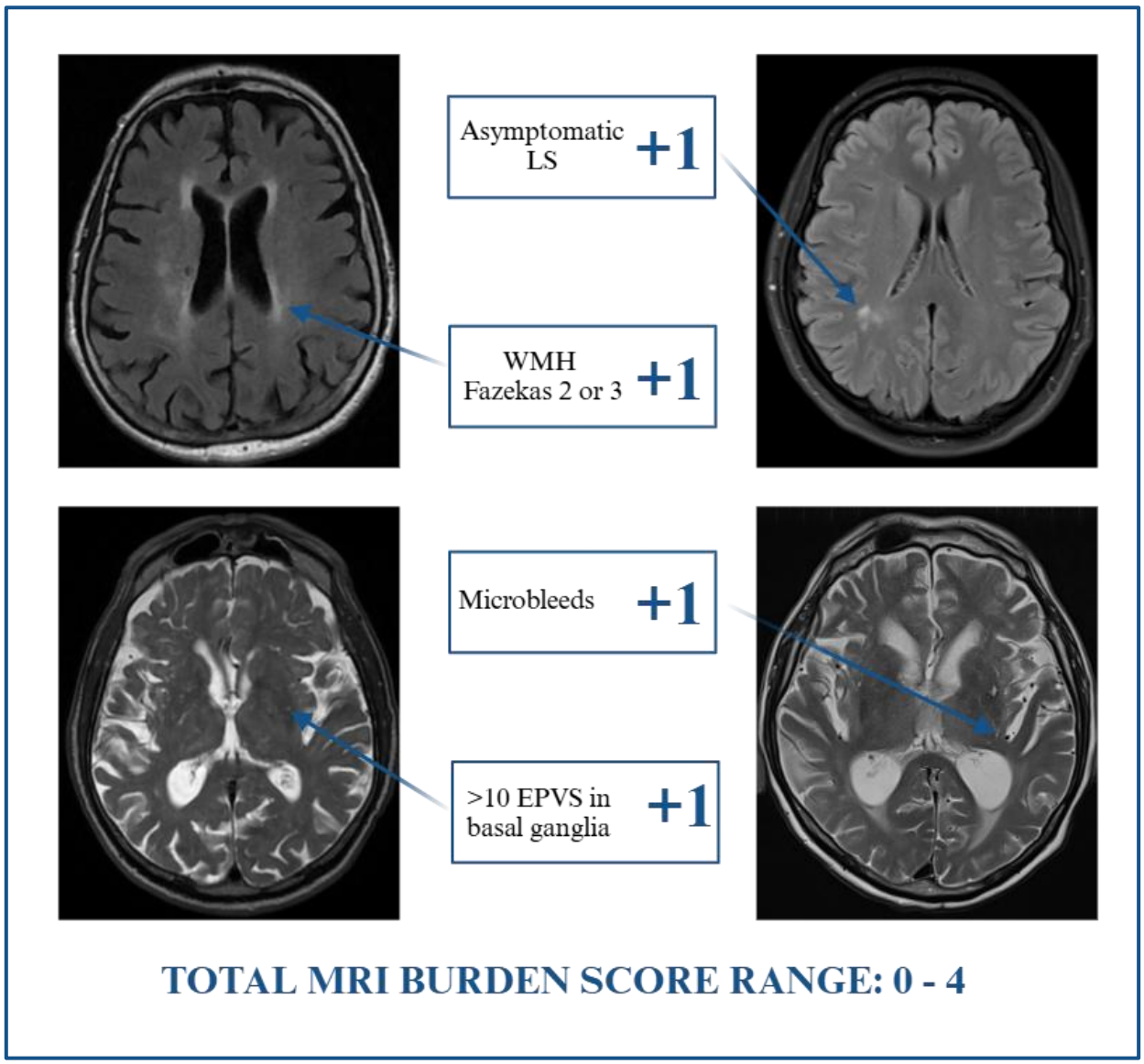

Figure 4. CSVD MRI lesion burden scoring. Abbreviations: LS – lacunar strokes, WMH – white matter hyperintensities, EPVS – enlarged perivascular spaces. Created in Biorender.com

1.5.2. Biomarkers

Several biomarkers of CSVD have been proposed, but none have been used clinically so far. Currently, biomarkers for CSVD are used only for research purposes. The first obvious choice is the CSF to serum albumin ratio as a marker of BBB dysfunction, which showed an association with WMH and the CSVD MRI burden score (57–59). Next, inflammatory markers have been examined. C-reactive protein (CRP) was examined by several studies as a potential biomarker due to its relationship to endothelial dysfunction. It was found that CRP levels correlated with severe CSVD. Other inflammatory markers of interest included interleukin (IL) 6 and IL-1β, both of which were related to the risk of vascular events in CSVD patients (99). Systemic inflammation has been recognized as a risk factor for CSVD. Therefore, NLR was suggested as a potential biomarker. In CSVD, elevated NLR has been associated with disease progression, MRI burden, and cognitive impairment (60–62). However, the neutrophil count on its own has shown a good correlation with CSVD lesions (100).

As mentioned before, hyperhomocysteinemia was established as a risk factor for CSVD, making homocysteine another potential biomarker (69,70,99). Among other markers, some studies

evaluated the use of alkaline phosphatase (ALP). The authors found that it correlated with the development of depression in CSVD patients, but noted that the diagnostic performance improves when combined with hemoglobin, CRP, and HDL-C (101). Coagulation factors have been recently investigated in CSVD. Xu et al. found that D-dimer and fibrinogen increase correlated with the increase in WMH severity, although the authors didn't propose a cause for this correlation. Other studies also reported this correlation, with some noting that the von Willebrand Factor had a negative correlation with EPVS, and its low levels could indicate impaired endothelial integrity in the brain (99,102).

1.5.3. Evaluating atherosclerosis

Due to the correlation between arteriolosclerotic CSVD and large artery atherosclerosis, recent studies have begun investigating the degree of large artery atherosclerosis in CSVD patients using ultrasonography or MRI imaging. Wang et al. showed a correlation between intracranial atherosclerosis, while Shu et al. found the same correlation using magnetic resonance angiography (MRA), indicating a place for MRA in the evaluation of CSVD patients (44,103). Other authors used MRI to assess the composition of carotid plaques and correlate plaque features (intraplaque hemorrhage) with the CSVD disease burden (33). The correlation between ICA stenosis and the CSVD MRI burden score has been previously described, even in cases of low-grade stenosis of less than 50% (84,104). Carotid stenosis in correlation to LS has been evaluated in a large number of studies with variable results. Some authors show that the presence of significant carotid stenosis and LS at the same time is purely incidental. Others point out that carotid artery stenosis in LS is an important marker of atherosclerosis without a necessary causal effect (105–107). With these findings, we can conclude that CSVD patients might benefit from extracranial artery ultrasonography examination, which can help not only assess atherosclerosis but also potentially identify the cause of stroke in patients with symptomatic LS.

1.6. Treatment of arteriolosclerotic cerebral small vessel disease

Currently, there is no active treatment for CSVD. This could possibly stem from the inadequate understanding of CSVD pathology, and the fact that CSVD is usually a silent and chronic disease (7).

1.6.1. Acute treatment

In acute settings, tissue plasminogen activator (tPA) has been investigated for CSVD treatment. tPA is considered a gold standard for the treatment of acute ischemic stroke (AIS) but has shown variable results in LS. One study showed that patients with LS who received tPA had better outcomes than the placebo group. It is important to note that the presence of WMH and microbleeds increased the risk of intraparenchymal hemorrhage by 50% (108). More recent studies also showed the benefits of tPA in patients with acute symptomatic LS (109,110). However, there is still debate whether microbleeds should be considered a contraindication for tPA or not (1,7).

Another treatment used for AIS involves antiplatelets to prevent a secondary stroke (1). One pooled analysis of 17 trials assessed the benefit of antiplatelet therapy in patients with acute LS. They found that single antiplatelet therapy (SAPT) was adequate to prevent a secondary stroke. However, dual antiplatelet therapy (DAPT) didn't show additional benefits when compared to SAPT (111). This could stem from the CSVD contributing to clopidogrel resistance. A study by Lundström and associates showed that resistance to clopidogrel was more common in patients with moderate and severe CSVD than in those with no CSVD or mild disease. The authors attempted to attribute this finding to CSVD pathology causing endothelial dysfunction that increases platelet aggregation, and

shear activation of platelets that is more common in small stenotic arteries (112). Additionally, the SPS3 clinical trial showed that DAPT with aspirin and clopidogrel didn't reduce the risk of recurrent AIS in LS patients. However, this combination increased the risk of bleeding significantly (113). Recent studies identified cilostazol as a superior antiplatelet agent for patients with acute LS. A large meta-analysis noted that cilostazol could be a superior option when compared to placebo, aspirin, clopidogrel, and DAPT with aspirin and clopidogrel or aspirin and dipyridamole (114). A study from Nishiyama et al. analyzed DAPT vs SAPT and found that the DAPT combination of cilostazol and aspirin was superior to SAPT (115). Finally, Nakamura et al. showed that patients treated with this combination in the acute phase of any non-cardioembolic AIS had better functional status (116). According to European Stroke Organization (ESO) guidelines on covert (asymptomatic) CSVD, antiplatelets are not recommended for covert CSVD and could be harmful in older patients (117).

1.6.2. Chronic treatment

Chronic treatment of CSVD is geared towards prevention by addressing modifiable risk factors (1). Hypertension, being the most important risk factor for CSVD, is a clear target for treatment. The SPRINT-MIND clinical trial assessed the effect of blood pressure management in individuals over the age of 50. The authors showed that intensive blood pressure management with a goal of systolic blood pressure of < 120 mmHg led to a smaller increase in WMH and decreased the incidence of mild cognitive impairment (MCI) when compared to the control group (118). These findings were confirmed by another clinical trial, the INFINITY study, while the ACCORD-MIND study placed importance on treating hypertension in patients with type 2 DM to reduce WMH progression (119,120). A post hoc analysis of data from the SPRINT-MIND study showed that blood pressure control didn't have the desired effect on cognition and cerebral perfusion in patients whose diastolic blood pressure was low at baseline. The authors note that reducing blood pressure in patients with low diastolic blood pressure might negatively affect brain perfusion (121). The use of antihypertensive medications in CSVD patients is recommended by ESO (117).

DM is another important risk factor for CSVD and dementia that can be targeted with treatment. Even though glycemic control has proven beneficial in the prevention of secondary stroke, it hasn't shown the desired effect on CSVD pathology (40,120,122). However, ESO recommends the use of glucose-lowering medications in patients with elevated glucose levels (117). Recent studies identified sodium-glucose cotransporter 1 and 2 (SGLT1, SGLT2) inhibitors as candidates to slow down CSVD progression. It is thought that this mechanism is not achieved by pure glycemic control but by SGLT1 and SGLT2 having an effect on the neurovascular unit and tight junctions of endothelial cells, thereby helping maintain the BBB (123).

Statins are another option for the treatment of CSVD. Besides lowering lipids, statins act anti-inflammatory and protectively on the endothelium (7). In CSVD, statins have been shown to decrease WMH progression and cognitive decline, next to decreasing the risk of stroke (124–126). Guo et al. showed that statins reduced the progression of WMH, lacunes, and EPVS, but not microbleeds (127). This could be due to the fact that WMH, lacunes, and EPVS are ischemic lesions, while microbleeds are hemorrhagic ones. Also, statin use has been shown to be associated with subcortical microbleeds in patients with intracerebral hemorrhage (128). ESO notes that there is not enough evidence to make a recommendation on the use of statins in covert CSVD (117). Smoking cessation is also suggested for patients with CSVD, as well as physical exercise (129). According to ESO, even though there is not enough evidence to recommend lifestyle modifications, it is reasonable to address these factors and promote healthy lifestyles in patients with covert CSVD (117).

1.7. Prognosis of cerebral small vessel disease

CSVD is usually a silent and chronic disease, mostly affecting the elderly. Even though the condition might be asymptomatic and diagnosed incidentally, it is an important contributor to dementia and ischemic stroke (6,7).

1.7.1. Large artery acute ischemic stroke

Just the presence of CSVD-related brain lesions increases the risk of stroke (27). This fact is reasonable as CSVD shares the same risk factors as a large artery acute ischemic stroke (LAAIS). Hypertension, DM, dyslipidemia, and smoking are as frequent in LS as in other ischemic strokes. (130,131). Carotid artery stenosis is more frequent in patients with LAAIS, but its correlation with CSVD is highly probable (130). It is not only the "traditional" cerebrovascular risk factors that are shared between these two conditions, but the "non-traditional" ones as well. For example, hyperhomocysteinemia is a known risk factor for stroke (69). Additionally, the same markers of systemic inflammation, like the NLR, that are suggested for CSVD, have been suggested to predict outcomes after AIS (132,133). Both kidney dysfunction and NAFLD have a high prevalence in patients with AIS (134,135). In patients with larger artery atherosclerosis, CSVD increases the risk of recurrent stroke (136,137). The disease burden also correlated with the occurrence, meaning the more CSVD markers on imaging are found, the greater the risk of recurrent stroke is (137). In fact, Han et al. showed that CSVD MRI burden has a stronger impact on AIS occurrence in the 4-year follow-up than large artery stenosis, including intracranial and carotid arteries (34). Additionally, CSVD patients have poorer long-term survival after an LAAIS and a higher risk of cardiac death (138).

CSVD also affects the treatment of large artery disease. In the case of endarterectomy, patients with CSVD imaging markers have a higher risk of cardiovascular death, independently of other risk factors. The association of CSVD, specifically WMH, and post-endarterectomy stroke is vague, with some studies reporting an increased 30-day stroke risk, while others didn't find this correlation (139,140). However, WMH have been shown to be an important risk factor for recurrent stroke in patients undergoing carotid artery stenting (141).

2. OBJECTIVES

Objectives of this study included:

1. The involvement of large artery atherosclerosis in CSVD

Determine the incidence and degree of large vessel atherosclerosis, large vessel function, and anatomical variations (angulation, kinking, coiling) in CSVD patients with and without LAAIS, and assess for differences between the two groups.

2. Correlation between the CSVD MRI burden and LAAIS

Determine the difference in disease burden on imaging (LS, WMH, microbleeds, EPVS, brain atrophy) in CSVD patients with and without LAAIS.

3. Assessment of "traditional" vascular risk factors for LAAIS in CSVD

Assess the sociodemographic characteristics and the prevalence of known vascular risk factors (dyslipidemia, hypertension, diabetes, smoking status) in CSVD patients and compare the differences between CSVD patients with and without LAAIS.

4. Assessment of "non-traditional" vascular risk factors for LAAIS in CSVD

Assess the presence of novel biomarkers and lesser-known risk factors (vitamin B12, folic acid, homocysteine, coagulation factors, liver function tests, kidney function tests, thyroid hormones, inflammation markers, BBB dysfunction markers) in CSVD patients and compare the differences between CSVD patients with and without LAAIS.

5. Influence of risk factors on the occurrence of LAAIS in CSVD patients

Determine the influence of sociodemographic and clinical parameters on the occurrence of LAAIS in patients with CSVD by calculating odds ratios (OR) and relative risk (RR).

3. MATERIAL AND METHODS

3.1. Study design

Patients treated at the Clinic for Neurology, University Clinical Center of Serbia, between 2014. and 2024. were included in this cross-sectional study. The study was conducted following the principles of the Declaration of Helsinki. The study was approved by the Ethical Committee of the University of Belgrade, Faculty of Medicine on the 22nd of May 2024. (number 25/V-17). Additional approval was requested from the Ethical Committee of the University Clinical Center of Serbia after study completion and granted on the 29th of January 2026. (number 95/60).

3.2. Participants

Approximately 300 patients treated at the Clinic for Neurology, University Clinical Center of Serbia, from 2014. to 2024. were initially considered for the study. Only patients with arteriolosclerotic CSVD were included in the study, therefore patients with other etiologies of CSVD (amyloid-related, genetic, inflammatory, and immunologically mediated CSVD) were excluded. Other exclusion criteria included the presence of space-occupying brain lesions (e.g. tumors) due to the possibility of these lesions mimicking AIS symptoms and causing ischemia on their own; the presence of hemorrhagic stroke; AIS caused by other etiology (TOAST 2 – cardioembolic); and insufficient data. Thirty-seven patients were excluded due to the presence of non-atherosclerotic CSVD, 3 had space-occupying brain lesions, 3 had a hemorrhagic stroke, 3 patients had an ischemic stroke caused by another etiology, and 21 patients were excluded due to insufficient data.

After applying exclusion criteria, the study included 241 patients. The participants were classified as those without LAAIS (including TOAST 3, 4, or 5) and those with LAAIS (including TOAST 1) based on imaging findings. The group of arteriolosclerotic CSVD patients without LAAIS included 178 patients, while the group of CSVD patients with LAAIS included 63 patients. The entire study protocol is shown in Figure 5.

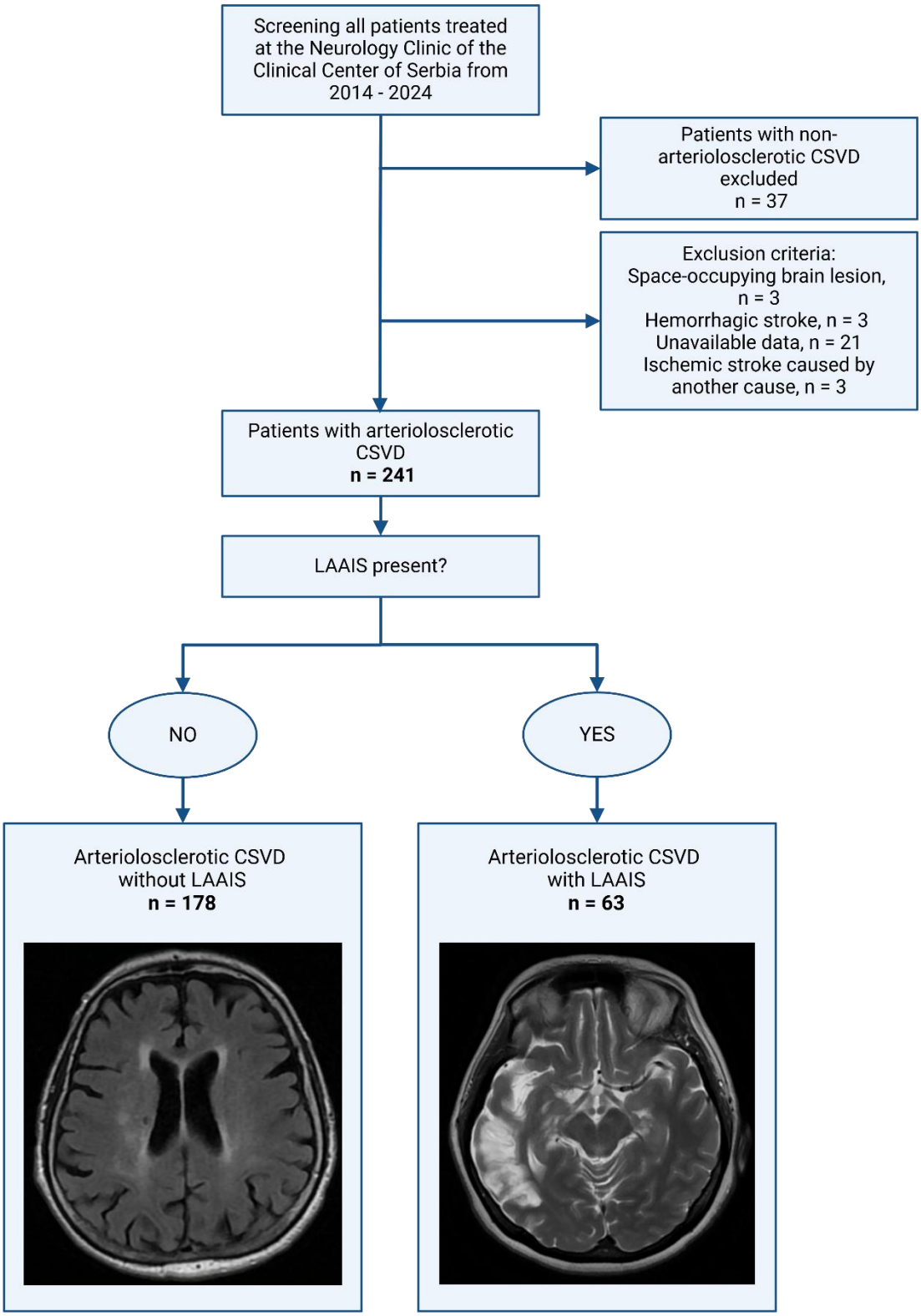

Figure 5. Study protocol showing exclusion criteria. Abbreviations: CSVD – cerebral small vessel disease, LAAIS – large artery acute ischemic stroke. Created in BioRender.com

3.3. Methods

3.3.1. Clinical data

Patients' medical documentation was evaluated, and relevant data were noted using the hospital information systems "Infomedis" and "Heliant". In the case of the acute event, data was obtained from the timepoint in the patient treatment and follow-up furthest from the event to avoid acute elevations of laboratory values and other findings influencing the analysis. The following data were noted for the purposes of this study:

1. Demographic data, including **age** and **sex.**
2. Clinical presentation, including **NIHSS** (Supplementary Figure 1) (142) at admission, previous **TIA**, and **ABCD2** score (Supplementary Figure 2) (143,144), the presence of other **atherosclerotic cardiovascular disease (CVD)** (CAD and peripheral arterial disease - PAD), the presence of **kidney dysfunction** (defined as reduced eGFR, or the presence of proteins in the urine without a urinary tract infection), if the patient had **LAAIS**, which side was involved, and what artery.
3. **TOAST** mechanism.
4. Presence and the type of **dyslipidemia**, laboratory values (total cholesterol, triglycerides, LDL-C, HDL-C); **DM** (glycosylated hemoglobin A1c - HbA1c); **hypertension**; and **smoking** status.
5. **Laboratory results of potential biomarkers** (urea, creatinine, eGFR, presence of protein in urine, protein level in blood, albumin, vitamin B12, folic acid, homocysteine, ALP, aspartate aminotransferase - AST, alanine aminotransferase - ALT, GGT, CRP, TSH, fT4, and free triiodothyronine - fT3).
6. Laboratory results related to **coagulation** (fibrinogen, D-dimer, antithrombin, plasminogen, protein C, prothrombin time - PT, partial thromboplastin time - aPTT, international normalized ratio – INR, coagulation factors II, V, VII, VIII, IX, X, XI, XII, XIII, lupus anticoagulant, and Von Willebrand factor).
7. **Complete blood count (CBC)** results (red blood cells - RBC, white blood cells - WBC, neutrophils, lymphocytes, thrombocytes, and hemoglobin values).
8. **Cerebrospinal fluid (CSF)** analysis results (glucose, albumin, number and type of cells present).

Reference ranges for each laboratory parameter are shown in Supplementary Table 1. Based on the collected data the following parameters were calculated:

1. BBB dysfunction parameter – **CSF to serum albumin ratio** was used as a parameter of BBB dysfunction, as it reflects the leakage of the BBB. The CSF/serum albumin ratio was calculated by dividing CSF albumin values by serum albumin values and multiplying the result by 1000.

CSF to serum albumin ratio = CSF albumin (g/L) / serum albumin (g/L) * 1000

Normal CSF/serum albumin ratio values were determined according to the patient's age based on previous studies. For patients ages 15-60, the normal ratio was between 5 and 8. For patients older than 60, the normal ratio ranged from 8-10 (57,58).

2. Marker of systemic inflammation – **blood neutrophil to lymphocyte ratio (NLR)** was used as a parameter of systemic inflammation. To calculate the NLR, we used the numbers of neutrophils and leukocytes from the CBC, measured from the venous blood samples. The following formula was used:

   NLR = neutrophil count (10^9/L) / leukocyte count (10^9/L)

   Values between 0.7 and 2.3 were considered normal; the range from 2.3 to 3.0 was defined as increasing, and anything above 3.0 was considered elevated based on previous studies (60–62).

3.3.2. Neuroimaging collection and interpretation

All patients in this study had neuroimaging-verified CSVD by CT and/or MRI. Standardized MRI protocol was used for all patients, and images were obtained using the 1.5 T Signa Artist (GE Healthcare, United States) scanner. We obtained standard axial T1-weighted, T2-weighted, FLAIR, susceptibility-weighted, and diffusion-weighted images. In the sagittal plane, we obtained T2-weighted images, and in the coronal plane, FLAIR was used. On CT, LS was defined as small hypodensity, and WMH were defined as hypodense periventricular lesions (5).

The following markers of CSVD on MRI were identified based on STRIVE criteria and noted:

1. **LS**, defined as recent subcortical infarct visible as hypointense lesions on T1-weighted MRI, or hyperintense lesions on T2-weighted and FLAIR MRI up to 20mm in size on axial sections; and lacunes, defined as round subcortical cavities with location corresponding to a previous LS, visible as hypointense lesions on T1-weighted MRI, hyperintense lesions on T2-weighted MRI, or with CSF-like intensity on FLAIR MRI, 3 to 15 mm in size. LS and lacunes were additionally classified as being present in the left, right, or both hemispheres.
2. **WMH**, defined as bilateral patchy or diffuse lesions in the periventricular white matter, visible as hypointensities on T1-weighted MRI, or hyperintensities on T2-weighted and FLAIR MRI. Additionally, WMH were graded according to the **modified Fazekas scale** (97). Grade 1 was defined as a thin lining around lateral ventricles; Grade 2 as a smooth halo around lateral ventricles; and Grade 3 as hyperintense lesions infiltrating into the deep white matter from lateral ventricles (Supplementary Figure 3).
3. **EPVS**, defined as fluid-filled spaces that correspond to penetrating arteries, seen as linear lesions when viewed parallel to the vessel or round when viewed perpendicular to the vessel, up to 3mm in size. EPVS have an intensity similar to CSF on all MRI sequences (T1-, T2-weighted, and FLAIR).
4. **Microbleeds**, defined as small hypointense lesions visible only on T2-weighted GRE MRI, 3 to 5 mm in size.
5. **Brain atrophy**, defined as lower cerebral volume visible on all MRI sequences, not corresponding to focal lesions (e.g., ischemia). Low volume was deduced from the enlargement of CSF spaces (sulci and ventricles) (6).

We calculated the CSVD MRI burden score based on the collected data regarding neuroimaging markers as described in previous studies (Figure 4). One point was given if there were one or more asymptomatic lacunar strokes (lacunes); One point for WMH Fazekas Grade 2 or 3; One point in the case of 1 or more microbleeds; and 1 point for the presence of more than 10 EPVS in the basal ganglia. The total point score, ranging from 0 to 4, was calculated for each patient (98).

LAAIS was defined as a large artery stroke, confirmed by CT and/or MRI. On CT, signs of LAAIS included loss of grey-white matter differentiation and a hypodense lesion. On MRI, LAAIS was defined as a hyperintense lesion on diffusion-weighted imaging (DWI) in case of an acute lesion,

and a hyperintense lesion on DWI, T2-weighted, and FLAIR MRI in case of subacute and chronic lesions. LAAIS was further classified based on the vascular territory it encompasses (ACA, MCA, PCA, and vertebrobasilar - VB territory) and side (left or right hemisphere) (145).

3.3.3. Extracranial color duplex sonography collection and interpretation

Large extracranial vessels (ICA and VA) were examined with extracranial color duplex sonography by specially trained neurologists. This examination was performed on all patients in our cohort. The Aloka Prosound Alpha 10 (Aloka, Japan) ultrasound machine with a linear ultrasound probe 5-13 MHz in the standardized protocol was used. The following morphological and hemodynamic parameters were recorded:

1. **The presence of plaques in carotid arteries** and their characteristics, as well as the **carotid diameter stenosis degree**, expressed in percentage according to the combined criteria (146). Based on the diameter stenosis percentage, we classified carotid artery stenosis as no stenosis in cases where it was 0%, low-grade stenosis ranging from 1-49%, and high-grade stenosis of 50% or more.
2. **Carotid stenosis sum**, defined as the summation of the carotid diameter stenosis of the left and right carotid artery.
3. **Intima-media thickness (IMT**) in the left and the right common carotid artery, measured in millimeters, 2 cm from the carotid bifurcation, according to the Mannheim consensus (147).
4. **IMT sum**, defined as the summation of IMT values of the left and right carotid artery.
5. **Anatomic variations** found in ICA (angulation, kinking, and coiling).
6. ICA hemodynamic parameters, including **peak systolic velocity (PSV)** and **end-diastolic velocity (EDV)**. Hemodynamic parameters were calculated automatically by the ultrasound machine, and values for PSV and in the proximal part of the ICA were recorded during the exam in cm/s.
7. **VA characteristics**, defined as the **presence of plaque, hypoplasia, and low flow**, as well as **VA PSV and EDV** in V2 segments of the vertebral arteries. Hemodynamic parameters were calculated in the same way as for ICA.

3.3.4. Transcranial color Doppler sonography collection and interpretation

Transcranial color Doppler (TCD) examination was performed by specially trained neurologists using the pulsed Doppler probe 2MHz TCD (Rimed Ltd, Israel) through the transtemporal and transforaminal insonation windows. MCA was insonated at the depth of 45-65mm; ACA at the depth of 60-75mm; PCA at the depth of 60-75mm; VA at the depth of 65-85mm; and BA was insonated at the depth of 90-120mm (148).

Mean flow velocity (MFV) was calculated for each vessel using the following formula:

$$\mathbf{MFV} = (PSV + EDV \times 2) / 3$$

Intracranial vessel stenosis was defined based on the MFV values. The following MFV values were considered indicative of intracranial vessel stenosis of >50%: MCA >100cm/s, ACA >80cm/s, PCA>80cm/s, VA>90cm/s, BA>90cm/s (149).

The pulsatility index (PI) for each vessel was calculated using flow velocity (FV) in the following formula:

PI = (systolic FV – diastolic FV) / mean FV

Normal PI ranged from 0.6 to 1.1. Increased values were considered indicative of significant stenosis or occlusion, while decreased values were considered indicative of low flow as compensation for reduced perfusion. Both MFV and PI were calculated automatically by the ultrasound machine and recorded during examination in cm/s (150).

3.3.5. Cerebral vasomotor reactivity collection and interpretation

We used the breath-holding index (BHI) to measure cerebral vasomotor reactivity (VMR). The measurement was performed on both MCA (left and right) through the temporal bone window at a depth of 55mm, using a TCD ultrasonic transducer 2MHz (Rimed Ltd, Israel). Patients were investigated in a supine position. They were instructed to perform a normal inhalation and hold their breath for 30 seconds. Arterial blood pressure and heart rate were continuously measured during the test. Ultrasonic transducers (2MHz) were set on the left and the right side of the temporal bone to continuously measure MFV and PI in both MCAs. Insonation depth was 50-60mm based on the present factors (usually 55mm).

BHI was calculated using a standardized formula:

BHI = (MFV at the end of apnea – baseline MFV) / baseline MFV x (100 / seconds of apnea)

We considered a BHI value of at least 0.69 to be normal. In cases when BHI was less than 0.69, we noted it as pathological, therefore, cerebral VMR was marked as decreased (151).

3.4. Statistical analysis

The Kolmogorov-Smirnov $\chi 2$-test was used to determine the normality of distribution among variables in the cohort. Based on the results, it was determined that no variable had a normal distribution, and appropriate statistical tests were chosen. Variables were adjusted for the effect of age and sex when appropriate (i.e., variables that are known to be affected by sex, such as RBC, platelets, hemoglobin, and folic acid levels). The adjustment was based on the underlying fitting of regression models, returning residual values of the regression models. To test for differences between groups, Pearson's chi-square test and Fisher's exact test were used for nominal variables. For ordinal variables and numeric variables (non-normal distribution), the Wilcoxon rank sum test and the Kruskal-Wallis rank sum test were used. Data is presented in tables showing numeric (n) values and percentages in the case of nominal and ordinal variables, and median with interquartile range (IQR) in the case of numeric variables, and p values of adjusted data. Multivariate logistic regression was performed using variables that showed statistically significant differences between groups as predictors adjusted for age and sex, and the occurrence of LAAIS as a response variable. OR was calculated from the regression analysis using the established formula OR = exp(regression coefficient) (152). The results of logistic regression are shown in a table and graphically as OR with 95% confidence interval (CI) and p values. RR was calculated based on the following formula: incidence in the exposed group (exposed with event / all exposed) / incidence in the non-exposed group (non-exposed with event /

all non-exposed). OR and RR cutoff of 1 was used, meaning that values above 1 indicated that the odds/risk of the event are increased and those with values below 1 indicated that the odds/risk of the event are decreased, while the OR/RR of 1 indicated that the odds/risk of the event don't change relative to the predictor. For all statistical tests, p value of less than or equal to 0.05 was considered statistically significant. R studio (version 4.3.2) was used to perform all statistical analyses (153). Packages gtsummary, datawizard, and desctools were used for statistical analysis and to create tables, and package ggplot2 was used to create figures (154–157).

4. RESULTS

4.1. Sociodemographic characteristics and risk factor profile of patients with arteriolosclerotic cerebral small vessel disease

The study cohort included 241 patients with type 1 arteriolosclerotic CSVD. Data availability varied for each variable (Supplementary Table 2) due to data loss over time, as in the period from 2014 to 2024, record keeping switched from paper to electronic format, and then to a different hospital information system. In our cohort, patient age ranged from 22 to 90 (median age 65, IQR 56-73). There were 131 (54%) female patients and 110 (46%) males. Hypertension was found in 188 (78%) patients, while dyslipidemia was present in 141 (59%) patients. Seventy-three (30%) had DM, 87 (45%) were smokers, and 38 (16%) had kidney dysfunction. Other atherosclerotic CVD (most commonly myocardial ischemia) was found in 28 (12%) of patients. The total cholesterol median value was 4.74 (IQR 4.09-5.66). Median HDL-C and LDL-C were 1.46 (IQR 1.14-1.82) and 2.60 (IQR 2.00-3.23), respectively. The median value for triglycerides was 1.25 (IQR 0.99-1.86). Sociodemographic and cardiovascular risk factor-related data are shown in Table 1.

Table 1. Sociodemographic characteristics and distribution of risk factors in arteriolosclerotic CSVD patients

Characteristic	N = 241[1]
Age	65 (56 – 73)
Sex	
female	131 (54)
male	110 (46)
Hypertension	188 (78)
Dyslipidemia	141 (59)
DM	73 (30)
Smoker	87 (45)
Kidney dysfunction	38 (16)
Atherosclerotic CVD	28 (12)
Total cholesterol (mmol/L)	4.74 (4.09 – 5.66)
HDL-C (mmol/L)	1.46 (1.14 – 1.82)
LDL-C (mmol/L)	2.60 (2.00 – 3.23)
Triglycerides (mmol/L)	1.25 (0.99 – 1.86)

Abbreviations: DM – diabetes mellitus, CVD – cardiovascular disease, HDL-C – high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol. [1]Median (IQR); n (%)

All patients underwent color duplex examination of extracranial cerebral vessels. The morphological parameters of ICA are shown in Table 2. In the cohort, 172 (71%) had plaques in any ICA, out of which 159 (66%) had plaques in the right ICA, with a median stenosis of 30% (IQR 0-30). Out of them, 140 (58%) had low-grade stenosis and 19 (7.9%) had high-grade stenosis. On the left side, 155 (64%) patients had plaques, with a median value of 30% (IQR 0-30). Low-grade stenosis of the left ICA was found in 139 (58%) patients, while 16 (6.6%) had high-grade stenosis. Sum stenosis median was 50 (IQR 0-65). The right, left, and sum IMT values were 1.10 (IQR 0.9-1.3), 1.20 (IQR 0.9-1.3), and 2.3 (IQR 1.8-2.5), respectively. Anatomic variations of ICA, including kinking, coiling, or angulations, were present in 64 (27%) patients on the right side and 70 (29%) patients on the left side. The details on hemodynamic ICA parameters are shown in Table 3.

Table 2. Extracranial artery color duplex sonography examination morphological parameters of ICA in arteriolosclerotic CSVD patients

Characteristic	**N = 241**[1]
ICA plaque	172 (71)
Right ICA plaque	159 (66)
Right ICA stenosis (%)	30 (0 – 30)
Right ICA stenosis degree	
no stenosis	82 (34)
low-grade stenosis	140 (58)
high-grade stenosis	19 (7.9)
Left ICA plaque	155 (64)
Left ICA stenosis (%)	30 (0 – 30)
Left ICA stenosis degree	
no stenosis	86 (36)
low-grade stenosis	139 (58)
high-grade stenosis	16 (6.6)
Sum stenosis	50 (0 – 65)
Right IMT (mm)	1.10 (0.90 – 1.30)
Left IMT (mm)	1.20 (0.90 – 1.30)
Sum IMT	2.30 (1.80 – 2.50)
Right ICA variations	64 (27)
Left ICA variations	70 (29)

Abbreviations: ICA – internal carotid artery, IMT – intima-media thickness. [1]n (%); Median (IQR).

When it comes to VA, only 10 patients (4.1%) had right VA plaques, and 2 (0.8%) had right VA hypoplasia. On the left side, VA plaques were found in 7 (2.9%) patients, while hypoplasia was present in 1 (0.4%) patient. The morphological and hemodynamic parameters of VA are shown in Table 3.

Table 3. Extracranial artery color duplex sonography examination hemodynamic ICA parameters and morphological and hemodynamic VA parameters in arteriolosclerotic CSVD patients

Characteristic	N = 241[1]
Right ICA PSV	77 (68 – 82)
Right ICA EDV	29 (25 – 32)
Left ICA PSV	78 (70 – 88)
Left ICA EDV	30 (28 – 35)
Right VA plaque	10 (4.1)
Right VA hypoplasia	2 (0.8)
Left VA plaque	7 (2.9)
Left VA hypoplasia	1 (0.4)
Right VA PSV	40 (32 – 42)
Right VA EDV	18 (14 – 20)
Left VA PSV	40 (34 – 42)
Left VA EDV	17 (14 – 20)

Abbreviations: ICA – internal carotid artery, PSV – peak systolic velocity, EDV – end-diastolic velocity, VA – vertebral artery. [1]n (%); Median (IQR).

TCD examination was performed on 93 patients (Table 4). Anterior circulation stenosis was found in 12 (18%), while 12 (18%) had low flow. Stenosis in the VB circulation was present in 14 (15%) patients, and 25 (27%) had low flow. Hemodynamic parameters of ACA, MCA, PCA, VA, and BA are shown in Table 4. VMR testing was performed on 25 patients. Median BHI was 0.95 (IQR 0.83 – 1.19) in the right, and 0.98 (IQR 0.78 – 1.22) in the left MCA. Finally, low VMR was found in 2 (8%) patients on the right side and 4 (17%) on the left side.

Table 4. Transcranial color Doppler sonography findings in arteriolosclerotic CSVD patients

Characteristic	N = 93[1]
Anterior stenosis	12 (18)
Anterior low flow	12 (18)
VB stenosis	14 (15)
VB low flow	25 (27)
Right ACA MFV	43 (34 – 50)
Right ACA PI	0.80 (0.70 – 0.90)
Right MCA MFV	53 (45 – 61)
Right MCA PI	0.80 (0.70 – 0.90)
Right PCA MFV	39 (27 – 45)
Right PCA PI	0.80 (0.70 – 0.90)
Left ACA MFV	44 (35 – 50)
Left ACA PI	0.80 (0.70 – 0.90)
Left MCA MFV	55 (46 – 62)
Left MCA PI	0.80 (0.70 – 0.90)
Left PCA MFV	38 (28 – 44)
Left PCA PI	0.80 (0.70 – 0.90)
Right VA MFV	32 (26 – 38)
Right VA PI	0.80 (0.70 – 1.00)
Left VA MFV	32 (25 – 38)
Left VA PI	0.80 (0.70 – 1.00)
BA1 MFV	31 (24 – 40)
BA1 PI	0.80 (0.70 – 0.90)
BA2 MFV	31 (25 – 40)
BA2 PI	0.80 (0.70 – 1.00)
Right BHI	0.95 (0.83 – 1.19)
Left BHI	0.98 (0.78 – 1.22)
Low right VMR	2 (8)
Low left VMR	4 (17)

Abbreviations: VB – vertebrobasilar. ACA – anterior cerebral artery, MCA – middle cerebral artery, PCA – posterior cerebral artery, VA – vertebral artery, BA – basilar artery, MFV – mean flow velocity, PI – pulsatility index, BHI – breath-holding index, VMR – vasomotor reactivity. [1]n (%); Median (IQR).

All patients had neuroimaging-verified CSVD (Table 5). Forty-five had CT and 196 had MRI performed and assessed. LS were prevalent in our cohort and found in 217 (90%) patients. Most of these patients had bilateral lesions (n=192, 88%), while 12 (5.5%) had LS only in the right and 13 (6%) had LS only in the left hemisphere. WMH were found in 104 (43%) individuals. Only WMH on MRI were graded on the modified Fazekas scale. Twenty-seven (36%) had grade 1 lesions, 36 (48%) had grade 2, and 12 (16%) had grade 3 WMH. Only 17 (8.7%) patients had microbleeds. EPVS were present in 64 (33%) patients, out of which 19 (30%) had less than 10 EPVS, 31 (48%) had 10-25 EPVS, and 14 (22%) had more than 25 EPVS in the basal ganglia. The final marker of CSVD, brain atrophy, was found in 93 (39%) individuals. The total CSVD MRI burden score ranged from 0 to 3, without any patients scoring the maximal possible value of 4. Seventeen (8.7%) patients had a score of 0, and 126 (64%) had a score of 1. A score of 2 was found in 44 (22%) patients, while only 9 (4.6%) had a score of 3.

Table 5. Neuroimaging findings in arteriolosclerotic CSVD patients

Characteristic	**N = 241** [1]
Imaging	
CT	45 (19)
MRI	196 (81)
LS	217 (90)
LS localization	
right hemisphere	12 (5.5)
left hemisphere	13 (6)
bilateral	192 (88)
WMH	104 (43)
WMH Fazekas score	
grade 1	27 (36)
grade 2	36 (48)
grade 3	12 (16)
Microbleeds	17 (8.7)
EPVS	64 (33)
EPVS Category	
< 10 EPVS	19 (30)
10 - 25 EPVS	31 (48)
> 25 EPVS	14 (22)
Atrophy	93 (39)
CSVD MRI burden score	
score 0	17 (8.7)
score 1	126 (64)
score 2	44 (22)
score 3	9 (4.6)

Abbreviations: CT – computed tomography, MRI – magnetic resonance imaging, LS – lacunar strokes, WMH – white matter hyperintensities, EPVS – enlarged perivascular spaces, CSVD – cerebral small vessel disease. [1]n (%); Median (IQR)

Clinically, 116 patients presented acutely, with 63 (26%) having LAAIS (TOAST type 1) and 53 (22%) having symptomatic LS (TOAST type 3). Out of 63 patients with LAAIS, 21 (40%) had it in the right and 25 (48%) in the left brain hemisphere. The side was undefined for 6 patients as they had a stroke involving vertebrobasilar circulation (e.g., pons, medulla), which doesn't belong to a specific hemisphere. Data was missing for 11 patients. Out of 52 patients we had data on, the most commonly affected artery was the left MCA (n=16, 31%), followed by the right MCA (n=9, 17%). For all acute patients, the NIHSS median was 6 (IQR 5-8). Thirty-five (15%) out of all 241 patients reported having previous TIA, with a median ABCD2 score of 4 (IQR 3-5). Detailed clinical findings are shown in Table 6.

Table 6. Clinical findings in arteriolosclerotic CSVD patients who presented acutely

Characteristic	N = 116[1]
TOAST	
TOAST type 1	63 (26)
TOAST type 3	53 (22)
LAAIS	63 (26)
Side	
right hemisphere	21 (40)
left hemisphere	25 (48)
undefined	6 (12)
Artery	
right ACA	5 (9.6)
right MCA	9 (17)
right PCA	7 (13)
left ACA	4 (7.7)
left MCA	16 (31)
left PCA	5 (9.6)
VB	6 (12)
NIHSS	6 (5 – 8)
TIA	35 (15)
ABCD2	4 (3 – 5)

Abbreviations: TOAST - Trial of Org 10172 in acute stroke treatment, LAAIS – large artery acute ischemic stroke, ACA – anterior cerebral artery, MCA – middle cerebral artery, PCA – posterior cerebral artery, VB – vertebrobasilar, NIHSS - National Institutes of Health Stroke Scale, TIA – transient ischemic attack. [1]n (%); Median (IQR)

Median CBC values were 4.33 (IQR 4.03-4.63) for RBC, 6.90 (IQR 6.00-8.30) for WBC, 4.30 (IQR 3.50-5.48) for neutrophils, and 1.80 (IQR 1.40-2.20) for lymphocytes (Table 7). The NLR was calculated for 178 patients, and the median value was 2.39 (IQR 1.79-3.28). Normal NLR values were found in 83 (47%) patients, while only 1 (0.6%) had low values. Increasing values were discovered in 38 (21%) individuals, and 56 (31%) had an elevated NLR. The median platelet count was 230 (IQR 199-260). The median hemoglobin value was 136 (IQR 127-144). Median values with IQR for other laboratory findings, including glucose, HbA1c, urea, creatinine, eGFR, serum proteins, albumin, vitamin B12, folic acid, homocysteine, ALP, AST, ALT, GGT, CRP, TSH, fT3, and fT4, are shown in Table 8.

Table 7. Laboratory results including complete blood count parameters and neutrophil to lymphocyte ratio in arteriolosclerotic CSVD patients

Characteristic	**N = 241**[1]
RBC (10^12/L)	4.33 (4.03 – 4.63)
WBC (10^9/L)	6.90 (6.00 – 8.30)
Neutrophils (10^9/L)	4.30 (3.50 – 5.48)
Lymphocytes (10^9/L)	1.80 (1.40 – 2.20)
NLR	2.39 (1.79 – 3.28)
NLR groups	
low	1 (0.6)
normal	83 (47)
increasing	38 (21)
elevated	56 (31)
PLT (10^9/L)	230 (199 – 260)
Hgb (g/L)	136 (127 – 144)

Abbreviations: RBC – red blood cells, WBC – white blood cells, NLR – neutrophil to lymphocyte ratio, PLT – platelets, Hgb – hemoglobin. [1]Median (IQR); n (%)

Table 8. Laboratory results including the metabolic panel and potential biomarkers in arteriolosclerotic CSVD

Characteristic	N = 241[1]
HbA1c (%)	6.85 (5.68 – 8.00)
Urea (mmol/L)	6.10 (4.80 – 6.98)
Creatinine (µmol/L)	76 (67 – 94)
eGFR (mL/min/1.73m2)	60.0 (60.0 – 60.0)
Serum Proteins (g/L)	69.0 (67.0 – 71.0)
Albumin (g/L)	41.0 (40.0 – 43.0)
B12 (pg/mL)	371 (288 – 602)
Folic Acid (mmol/L)	5.5 (4.1 – 7.8)
Homocysteine (µmol/L)	12 (10 – 16)
ALP (U/L)	69 (60 – 79)
AST (U/L)	20 (17 – 23)
ALT (U/L)	27 (23 – 33)
GGT (U/L)	22 (18 – 27)
CRP (mg/L)	1 (1 – 5)
TSH (µIU/ml)	1.34 (0.97 – 1.81)
fT3 (pmol/L)	4.01 (3.81 – 4.39)
fT4 (pmol/L)	12.50 (11.40 – 13.83)

Abbreviations: HbA1c - glycosylated hemoglobin A1c, eGFR – estimated glomerular filtration rate, ALP – alkaline phosphatase, AST – aspartate aminotransferase, ALT – alanine aminotransferase, GGT – gamma-glutamyl transferase, CRP – C-reactive protein, TSH – thyroid-stimulating hormone, fT3 – free triiodothyronine, fT4 - free thyroxine. [1]Median (IQR); n (%)

In our cohort, the median fibrinogen and D-dimer were 3.5 (IQR 2.70-4.20) and 0.51 (IQR 0.36-0.86), respectively. Median PT, aPTT, and INR were 11.90 (IQR 11.30-12.50), 26.3 (IQR 24.7-28.7), and 1.00 (IQR 0.96-1.05), respectively. Detailed laboratory findings related to coagulation, including coagulation factors, are shown in Table 9.

Table 9. Laboratory results related to coagulation in arteriolosclerotic CSVD patients

Characteristic	**N = 241**[1]
Fibrinogen (g/L)	3.50 (2.70 – 4.20)
D-dimer (µg/ml)	0.51 (0.36 – 0.86)
Antithrombin (%)	100 (91 – 111)
Plasminogen (%)	125 (106 – 132)
Protein C (%)	115 (99 – 130)
PT (s)	11.90 (11.30 – 12.50)
aPTT (s)	26.3 (24.7 – 28.7)
INR	1.00 (0.96 – 1.05)
Factor II (%)	122 (114 – 145)
Factor V (%)	133 (118 – 149)
Factor VII (%)	110 (91 – 138)
Factor VIII (%)	147 (110 – 148)
Factor IX (%)	127 (110 – 148)
Factor X (%)	110 (102 – 129)
Factor XI (%)	134 (117 – 150)
Factor XII (%)	115 (92 – 135)
Factor XIII (%)	135 (125 – 154)
VonWillebrand Factor (%)	148 (100 – 157)
Positive lupus anticoagulant	8 (13)

Abbreviations: PT – prothrombin time, aPTT - partial thromboplastin time, INR – international normalized ratio. [1]Median (IQR); n (%)

The CSF analysis was performed in 83 patients (Table 10). The CSF glucose median was 3.50 (IQR 3.30-4.05), while CSF albumin had a median value of 0.46 (IQR 0.35-0.59). The CSF to serum albumin ratio was calculated for 82 patients, and the median value was 10.8 (IQR 8.8-14.1). Out of the 82, 59 (72%) patients had an elevated ratio, while in 23 (28%) the CSF to serum albumin ratio was not elevated.

Table 10. Cerebrospinal fluid analysis results in arteriolosclerotic CSVD patients

Characteristic	N = 83[1]
CSF Glucose (mmol/L)	3.50 (3.30 – 4.05)
CSF Albumin (g/L)	0.46 (0.35 – 0.59)
CSF PMN (n)	0 (0 – 0)
CSF RBC (n)	0 (0 – 30)
CSF WBC (n)	0 (0 – 1)
CSF/serum albumin ratio	10.8 (8.8 – 14.1)
CSF/serum albumin ratio groups	
not elevated	23 (28)
elevated	59 (72)

Abbreviations: CSF – cerebrospinal fluid, PMN – polymorphonuclear leukocytes, RBC – red blood cells, WBC – white blood cells. [1]Median (IQR); n (%)

4.2. Sex differences in patients with arteriolosclerotic cerebral small vessel disease

When assessing differences between male and female patients with arteriolosclerotic CSVD, we first adjusted the data for age as a confounding factor and then employed nonparametric tests as described in the statistics section, due to data distribution (Table 11). There was a statistically significant difference in sum stenosis (p=0.042), right (p=0.001), left (p=0.005), and sum IMT (p=0.002). In fact, women had lower levels in all 4 markers of ICA atherosclerosis. On imaging, women had a lower prevalence of microbleeds (3.7% vs 15%, p=0.005). Even though the prevalence of EPVS was the same, they were less numerous in women (p=0.021). Clinically, the ABCD2 score median was the same (6 for both), but the IQR range was greater in men (4-6, p=0.035). As for the cerebrovascular risk factors, total cholesterol was greater (4.95 vs 4.49, p<0.001), but HDL-C was lower in women (1.63 vs 1.22, p<0.001). Men were more commonly smokers (54% vs 37% women, p=0.017). When discussing laboratory results, there was a difference in RBC, platelets, and hemoglobin between sexes (p<0.001 for all). Hemoglobin levels also differed, with low hemoglobin (anemia) being more prevalent in men (32% vs 17% in women, p=0.01). Both urea and creatinine were higher in men (p=0.004 and p<0.001, respectively), which is confirmed by men having more kidney dysfunction (21% vs 11% in women, p=0.045). Folic acid values were higher and homocysteine values lower in women (p=0.025 and p=0.004, respectively), with fewer women having hyperhomocysteinemia (16% vs 24%, p=0.020). Lastly, men had higher levels of albumin in CSF (median 0.54 vs 0.40 in women, p<0.001). The CSF to serum albumin ratio was also higher in men (13.8 vs 9.4 in women, p<0.001), and men had a higher prevalence of elevated ratio (91% vs 58% in women, p=0.041).

Table 11. Sex differences in patients with arteriolosclerotic cerebral small vessel disease

Characteristic	Patient Sex		
	female, N = 131[1]	**male**, N = 110[1]	**p-value**[2]
Sum stenosis	50 (0 – 60)	60 (20 – 78)	**0.042**
Right IMT (mm)	1.10 (0.90 – 1.20)	1.20 (1.00 – 1.30)	**0.001**
Left IMT (mm)	1.10 (0.90 – 1.20)	1.20 (1.10 – 1.30)	**0.005**
Sum IMT	2.20 (1.80 – 2.40)	2.40 (2.03 – 2.60)	**0.002**
Microbleeds	4 (3.7)	13 (15)	**0.005**
EPVS Category			**0.021**
< 10 EPVS	15 (44)	4 (13)	
10 - 25 EPVS	12 (35)	19 (63)	
> 25 EPVS	7 (21)	7 (23)	
ABCD2	4 (3 – 5)	4.00 (4 – 6)	**0.035**
Total Cholesterol (mmol/L)	4.95 (4.34 – 5.93)	4.49 (3.82 – 5.16)	**<0.001**
HDL-C (mmol/L)	1.63 (1.32 – 2.02)	1.22 (1.07 – 1.52)	**<0.001**
Smoker	38 (37)	49 (54)	**0.017**
RBC (10^12/L)	4.16 (4.03 – 4.43)	4.51 (4.26 – 4.82)	**<0.001**
PLT (10^9/L)	241 (220 – 273)	210 (180 – 242)	**<0.001**
Hgb (g/L)	129 (123 – 137)	143 (136 – 150)	**<0.001**
Hgb levels			**0.010**
Low (anemia)	22 (17)	35 (32)	
Normal	108 (82)	74 (67)	
Elevated	1 (0.8)	1 (0.9)	
Urea (mmol/L)	5.70 (4.30 – 6.20)	6.20 (5.40 – 7.60)	**0.004**
Creatinine (µmol/L)	72 (61 – 78)	87 (76 – 109)	**<0.001**
Kidney dysfunction	15 (11)	23 (21)	**0.045**
Folic Acid (mmol/L)	5.9 (4.4 – 8.8)	5.1 (3.5 – 6.4)	**0.025**
Homocysteine (µmol/L)	11 (9 – 14)	13 (11 – 19)	**0.004**
Hyperhomocysteinemia	16 (23)	24 (42)	**0.020**
CSF albumin (g/L)	0.40 (0.33 – 0.50)	0.54 (0.46 – 0.62)	**<0.001**
CSF/serum albumin ratio	9.4 (7.9 – 11.6)	13.8 (10.5 – 16.0)	**<0.001**
Elevated CSF/serum albumin ratio	28 (58)	31 (91)	**0.041**

Abbreviations: IMT – intima-media thickness, EPVS – enlarged perivascular spaces, HDL-C – high-density lipoprotein cholesterol, RBC – red blood cells, PLT – platelets, Hgb – hemoglobin, CSF – cerebrospinal fluid. [1]Median (IQR); n (%). [2]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test. Data adjusted for age.

4.3. Differences based on the CSVD MRI burden score

Statistical analysis showed some differences between patients based on disease severity, defined by the CSVD MRI burden score. First, there was a difference in patients' ages, showing a trend of older patients having a greater burden score (p=0.018). Next, all morphological parameters of ICA stenosis showed differences among groups (Table 12). In fact, all morphological parameters point to the worsening of ICA atherosclerosis with an increasing disease burden on MRI (p<0.001 for all). Additionally, low VMR on the left side was more commonly found in patients with a higher disease burden (p=0.046).

Table 12. Differences in extracranial and intracranial atherosclerosis markers assessed with ultrasonography between CSVD patients based on the CSVD MRI burden score

Characteristic	CSVD MRI Burden Score				p-value[2]
	score 0, N = 17[1]	**score 1**, N = 126[1]	**score 2**, N = 44[1]	**score 3**, N = 9[1]	
Age	51 (46 – 67)	60 (52 – 70)	64 (60 – 76)	73 (61 – 79)	**0.018**
ICA plaque	5 (29)	79 (63)	39 (89)	9 (100)	**<0.001**
Right ICA plaque	1 (5.9)	72 (57)	37 (84)	9 (100)	**<0.001**
Right ICA stenosis (%)	0 (0 – 0)	20 (0 – 30)	30 (29 – 46)	70 (70 – 70)	**<0.001**
Right ICA stenosis degree					**<0.001**
no stenosis	16 (94)	53 (42)	7 (16)	0 (0)	
low-grade stenosis	1 (5.9)	71 (56)	34 (77)	2 (22)	
high-grade stenosis	0 (0)	2 (1.6)	3 (6.8)	7 (78)	
Left ICA plaque	5 (29)	67 (53)	34 (77)	9 (100)	**<0.001**
Left ICA stenosis (%)	0 (0 – 20)	20 (0 – 30)	30 (20 – 46)	50 (30 – 70)	**<0.001**
Left ICA stenosis degree					**<0.001**
no stenosis	12 (71)	58 (46)	10 (23)	0 (0)	
low-grade stenosis	5 (29)	66 (52)	30 (68)	5 (56)	
high-grade stenosis	0 (0)	2 (1.6)	4 (9.1)	4 (44)	
Sum stenosis	0 (0 – 20)	35 (0 – 60)	60 (50 – 80)	130 (100 – 150)	**<0.001**
Right IMT (mm)	0.90 (0.80 – 1.00)	1.10 (0.90 – 1.20)	1.20 (1.10 – 1.30)	1.30 (1.20 – 1.30)	**<0.001**
Left IMT (mm)	0.80 (0.80 – 1.10)	1.10 (0.90 – 1.20)	1.20 (1.10 – 1.30)	1.30 (1.20 – 1.30)	**<0.001**
Sum IMT	1.80 (1.60 – 2.10)	2.20 (1.70 – 2.40)	2.40 (2.20 – 2.60)	2.60 (2.40 – 2.60)	**<0.001**
Low left VMR	0 (0)	1 (6.3)	2 (50)	1 (100)	**0.046**

Abbreviations: CSVD – cerebral small vessel disease, MRI – magnetic resonance imaging, ICA – internal carotid artery, IMT – intima-media thickness, VMR – vasomotor reactivity. [1]n (%); Median (IQR). [2]Fisher's exact test; Kruskal-Wallis rank sum test. Data adjusted for age.

When it comes to neuroimaging findings, WMH were more prevalent in patients with higher burden scores (p<0.001), and they had a greater Fazekas grade (p=0.028). Microbleeds and EPVS were also more prevalent in these patients (p=0.003 and p<0.001, respectively), and they had more numerous EPVS in the basal ganglia (p<0.001). The final marker of CSVD on imaging, brain atrophy, was also more prevalent with increasing burden (p=0.041). Clinically, the prevalence of LAAIS went from 12% in patients with a burden score of 0 to 78% in those with a burden score of 3 (p<0.001). NIHSS increased from 4.0 to 8.5 between these groups (p=0.046), and the prevalence of hypertension also went up (p=0.028). The results of the statistical analysis regarding neuroimaging and clinical data are shown in Table 13.

Table 13. Differences in neuroimaging and clinical findings between arteriolosclerotic CSVD patients based on the CSVD MRI burden score

Characteristic	CSVD MRI Burden Score				p-value[2]
	score 0, N = 17[1]	**score 1**, N = 126[1]	**score 2**, N = 44[1]	**score 3**, N = 9[1]	
WMH	3 (18)	34 (27)	30 (68)	8 (89)	**<0.001**
WMH Fazekas score					**0.028**
grade 1	2 (67)	18 (53)	7 (23)	0 (0)	
grade 2	1 (33)	12 (35)	17 (57)	6 (75)	
grade 3	0 (0)	4 (12)	6 (20)	2 (25)	
Microbleeds	0 (0)	6 (4.8)	10 (23)	1 (11)	**0.003**
EPVS	4 (24)	33 (26)	18 (41)	9 (100)	**<0.001**
EPVS Category					**<0.001**
< 10 EPVS	4 (100)	14 (42)	1 (5.6)	0 (0)	
10 - 25 EPVS	0 (0)	12 (36)	13 (72)	6 (67)	
> 25 EPVS	0 (0)	7 (21)	4 (22)	3 (33)	
Atrophy	4 (24)	51 (40)	25 (57)	6 (67)	**0.041**
LAAIS	2 (12)	22 (17)	14 (32)	7 (78)	**<0.001**
NIHSS	4.0 (3.0 – 5.0)	6.5 (5.0 – 8.3)	7.0 (5.0 – 9.0)	8.5 (7.3 – 9.0)	**0.046**
Hypertension	9 (53)	96 (76)	37 (84)	9 (100)	**0.028**

Abbreviations: CSVD – cerebral small vessel disease, MRI – magnetic resonance imaging, WMH – white matter hyperintensities, EPVS – enlarged perivascular spaces, LAAIS – large artery acute ischemic stroke, NIHSS - National Institutes of Health Stroke Scale. [1]n (%); Median (IQR).
[2]Fisher's exact test; Kruskal-Wallis rank sum test. Data adjusted for age.

When looking at laboratory results, we found statistically significant differences in WBC (p=0.020) and neutrophils (p=0.002), as well as the NLR (p=0.002) and NLR groups (p<0.001), all showing a tendency to increase with the burden score. Urea showed increased values with a rising CSVD burden score (p=0.022). Lastly, the prevalence of fT3 values increased with the burden (p=0.037), and the p-value for hyperhomocysteinemia was exactly 0.05. All results regarding laboratory findings are shown in Table 14.

Table 14. Differences in laboratory findings between CSVD patients based on the CSVD MRI burden score

Characteristic	CSVD MRI Burden Score				p-value[2]
	score 0, N = 17[1]	**score 1**, N = 126[1]	**score 2**, N = 44[1]	**score 3**, N = 9[1]	
WBC (10^9/L)	6.60 (6.10 – 8.40)	6.90 (5.60 – 8.00)	7.60 (6.78 – 8.95)	7.10 (6.80 – 8.10)	**0.020**
Neutrophils (10^9/L)	3.60 (3.45 – 4.30)	4.10 (3.30 – 4.90)	5.00 (4.50 – 6.40)	4.00 (3.83 – 5.43)	**0.002**
NLR	1.89 (1.58 – 2.46)	2.19 (1.67 – 2.78)	3.20 (2.24 – 5.14)	2.56 (1.66 – 3.41)	**0.002**
NLR groups					**<0.001**
low	0 (0)	0 (0)	0 (0)	1 (13)	
normal	6 (55)	56 (57)	10 (30)	2 (25)	
increasing	3 (27)	24 (24)	4 (12)	2 (25)	
elevated	2 (18)	18 (18)	19 (58)	3 (38)	
Urea (mmol/L)	4.90 (3.60 – 5.70)	5.80 (4.40 – 6.30)	6.20 (5.30 – 7.85)	7.60 (6.20 – 8.20)	**0.022**
Hyperhomocysteinemia	1 (8.3)	22 (33)	8 (32)	5 (71)	0.050
fT3 (pmol/L)	4.53 (3.91 – 4.66)	4.01 (3.74 – 4.02)	4.01 (3.94 – 4.58)	7.01 (5.23 – 8.80)	**0.037**

Abbreviations: CSVD – cerebral small vessel disease, MRI – magnetic resonance imaging, WBC – white blood cells, NLR – neutrophil to lymphocyte ratio, fT3 - free triiodothyronine. [1]n (%); Median (IQR). [2]Fisher's exact test; Kruskal-Wallis rank sum test. Data adjusted for age and sex (red blood cells, platelets, hemoglobin, creatinine, folic acid).

4.4. Differences between cerebral small vessel disease patients with symptomatic lacunar stroke and large artery stroke

When assessing differences between patients who presented with acute symptoms, i.e., symptomatic LS (TOAST type 3) and LAAIS (TOAST type 1), there were statistically significant differences in atherosclerosis markers on ultrasonography (Table 15). Patients with LAAIS had a greater prevalence of plaques in the right and left ICA (p=0.008 for right, p=0.013 for left ICA), as well as stenosis and the stenosis degree. Median stenosis in the right and left ICA were 20 in LS patients and 30 in LAAIS patients (p<0.001 for right, and p=0.012 for left ICA). High-grade stenosis was more prevalent in patients with LAAIS in both ICA (p=0.004 for right, p=0.025 for left ICA). Sum stenosis was also greater in the LAAIS group (median 40 in LS vs 70 in LAAIS, p<0.001). Out of all markers of hemodynamics, only the right VA PI was statistically different among groups, showing higher values in patients with LAAIS (p=0.041).

Table 15. Differences in extracranial and intracranial atherosclerosis markers assessed with ultrasonography between patients with acute arteriolosclerotic CSVD based on the TOAST type

Characteristic	TOAST Type		p-value[2]
	TOAST 3, N = 53[1] **LS**	**TOAST 1**, N = 63[1] **LAAIS**	
Right ICA plaque	32 (60)	52 (83)	**0.008**
Right ICA stenosis (%)	20 (0 – 30)	30 (23 – 50)	**<0.001**
Right ICA stenosis degree			**0.004**
no stenosis	21 (40)	10 (16)	
low-grade stenosis	29 (55)	40 (63)	
high-grade stenosis	3 (5.7)	13 (21)	
Left ICA plaque	34 (64)	53 (84)	**0.013**
Left ICA stenosis (%)	20 (0 – 30)	30 (20 – 50)	**0.012**
Left ICA stenosis degree			**0.025**
no stenosis	18 (34)	10 (16)	
low-grade stenosis	32 (60)	42 (67)	
high-grade stenosis	3 (5.7)	11 (17)	
Sum stenosis	40 (0 – 60)	70 (43 – 100)	**<0.001**
Right VA PI	0.70 (0.63 – 0.80)	0.80 (0.78 – 1.10)	**0.041**

Abbreviations: TOAST - Trial of Org 10172 in acute stroke treatment, LS – lacunar stroke, LAAIS – large artery acute ischemic stroke, ICA – internal carotid artery, VA – vertebral artery, PI – pulsatility index, [1]n (%); Median (IQR). [2]Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test. Data adjusted for age and sex (red blood cells, platelets, hemoglobin, creatinine, folic acid).

No separate imaging marker showed differences between groups, but the overall CSVD MRI burden score was higher in the LAAIS group (p<0.001). The prevalence of atherosclerotic CVD was 5.7% in the LS group and 22% in the LAAIS group (p=0.012). Even though there was no difference in dyslipidemia prevalence, LAAIS patients had lower levels of total cholesterol (p=0.019). LAAIS patients also had a greater prevalence of DM (p=0.043). Neutrophils and the NLR were higher in the LAAIS group, and the same group had a higher prevalence of elevated NLR (p=0.013, p=0.012, p=0.011, respectively). Out of all markers of coagulation, only Factor VII showed a difference between groups (p=0.037). Imaging findings, clinical data, and laboratory value differences between groups are shown in Table 16.

Table 16. Differences in imaging, clinical, and laboratory data in patients with acute arteriolosclerotic CSVD based on the TOAST type

Characteristic	TOAST Type		p-value[2]
	TOAST 3, N = 53[1]	**TOAST 1**, N = 63[1]	
	LS	**LAAIS**	
CSVD MRI Burden Score			**<0.001**
score 0	9 (19)	2 (4.4)	
score 1	31 (66)	22 (49)	
score 2	7 (15)	14 (31)	
score 3	0 (0)	7 (16)	
NIHSS	5.00 (4.00 – 7.00)	7.50 (6.00 – 9.25)	**<0.001**
Atherosclerotic CVD	3 (5.7)	14 (22)	**0.012**
Total Cholesterol (mmol/L)	4.88 (4.26 – 5.73)	4.32 (3.78 – 5.10)	**0.019**
DM	11 (21)	24 (38)	**0.043**
Neutrophils (10^9/L)	4.30 (3.80 – 4.70)	4.70 (3.98 – 6.50)	**0.013**
NLR	2.24 (1.66 – 2.96)	3.01 (2.00 – 5.10)	**0.012**
NLR groups			**0.011**
low	0 (0)	1 (1.9)	
normal	22 (56)	13 (25)	
increasing	7 (18)	12 (23)	
elevated	10 (26)	26 (50)	
Factor VII (%)	125 (110 – 146)	102 (92 – 128)	**0.037**

Abbreviations: TOAST - Trial of Org 10172 in acute stroke treatment, LS – lacunar stroke, LAAIS – large artery acute ischemic stroke, CSVD – cerebral small vessel disease, MRI – magnetic resonance imaging, NIHSS - National Institutes of Health Stroke Scale, CVD – cardiovascular disease, DM – diabetes mellitus, NLR – neutrophil to lymphocyte ratio. [1]n (%); Median (IQR). [2]Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test. Data adjusted for age and sex (red blood cells, platelets, hemoglobin, creatinine, folic acid).

4.5. Frequency of risk factors among patients with arteriolosclerotic cerebral small vessel disease with and without consequential ischemic stroke

The results of statistical analysis comparing CSVD patients with LAAIS to those without are shown in Tables 17, 18, and 19. There were statistically significant differences in morphological markers of ICA atherosclerosis on ultrasonography (Table 17, Figure 6). Patients with LAAIS had a greater prevalence of plaques in any ICA (p=0.009 for any ICA plaque, p<0.001 for both left and right ICA plaques). Additionally, ICA stenosis was more severe in these patients (median 30% vs 20% stenosis in patients without LAAIS for both left and right side, p<0.001), which is confirmed by a greater prevalence of high-grade stenosis (p<0.001 for both sides) and a greater stenosis sum (p<0.001). The IMT on the right side and the sum IMT were also statistically different (p=0.008 and p=0.012, respectively). Due to the suspicion that these results are influenced by outliers, we excluded

all patients with ICA stenosis of more than 50% and repeated the analysis. All morphological markers of ICA atherosclerosis, except the presence of plaque in any ICA, were still statistically significant and showed an increased degree of ICA atherosclerosis among CSVD patients with LAAIS. There was also a difference in intracranial stenosis (Table 17). Patients with LAAIS had a greater prevalence of stenotic plaques in the anterior cerebral vasculature (38%) than those without LAAIS (12%, p=0.027). Another TCD finding, low VMR on the left side, was found in half of the patients with LAAIS, while in those without, the prevalence was 5.6% (p=0.035).

Table 17. Differences in extracranial and intracranial atherosclerosis markers assessed with ultrasonography between CSVD patients with and without large artery ischemic stroke

	CSVD and LAAIS		
Characteristic	**No LAAIS**, N = 178[1]	**Had LAAIS**, N = 63[1]	**p-value**[2]
ICA plaque	119 (67)	53 (84)	**0.009**
Right ICA plaque	106 (60)	52 (83)	**<0.001**
Right ICA stenosis (%)	20 (0 – 30)	30 (23 – 50)	**<0.001**
Right ICA stenosis degree			**<0.001**
no stenosis	72 (40)	10 (16)	
low-grade stenosis	100 (56)	40 (63)	
high-grade stenosis	6 (3.4)	13 (21)	
Left ICA plaque	101 (57)	53 (84)	**<0.001**
Left ICA stenosis (%)	20 (0 – 30)	30 (20 – 50)	**<0.001**
Left ICA stenosis degree			**<0.001**
no stenosis	76 (43)	10 (16)	
low-grade stenosis	97 (54)	42 (67)	
high-grade stenosis	5 (2.8)	11 (17)	
Sum stenosis	40 (0 – 60)	70 (43 – 100)	**<0.001**
Right IMT (mm)	1.10 (0.90 – 1.20)	1.20 (1.10 – 1.30)	**0.008**
Sum IMT (mm)	2.20 (1.80 – 2.40)	2.40 (2.15 – 2.60)	**0.012**
TCD anterior stenosis	6 (12)	6 (38)	**0.027**
Low left VMR	1 (5.6)	3 (50)	**0.035**

Abbreviations: CSVD – cerebral small vessel disease, LAAIS – large artery acute ischemic stroke, ICA – internal carotid artery, IMT – intima-media thickness, TCD – transcranial color Doppler, VMR – vasomotor reactivity. Data adjusted for age.

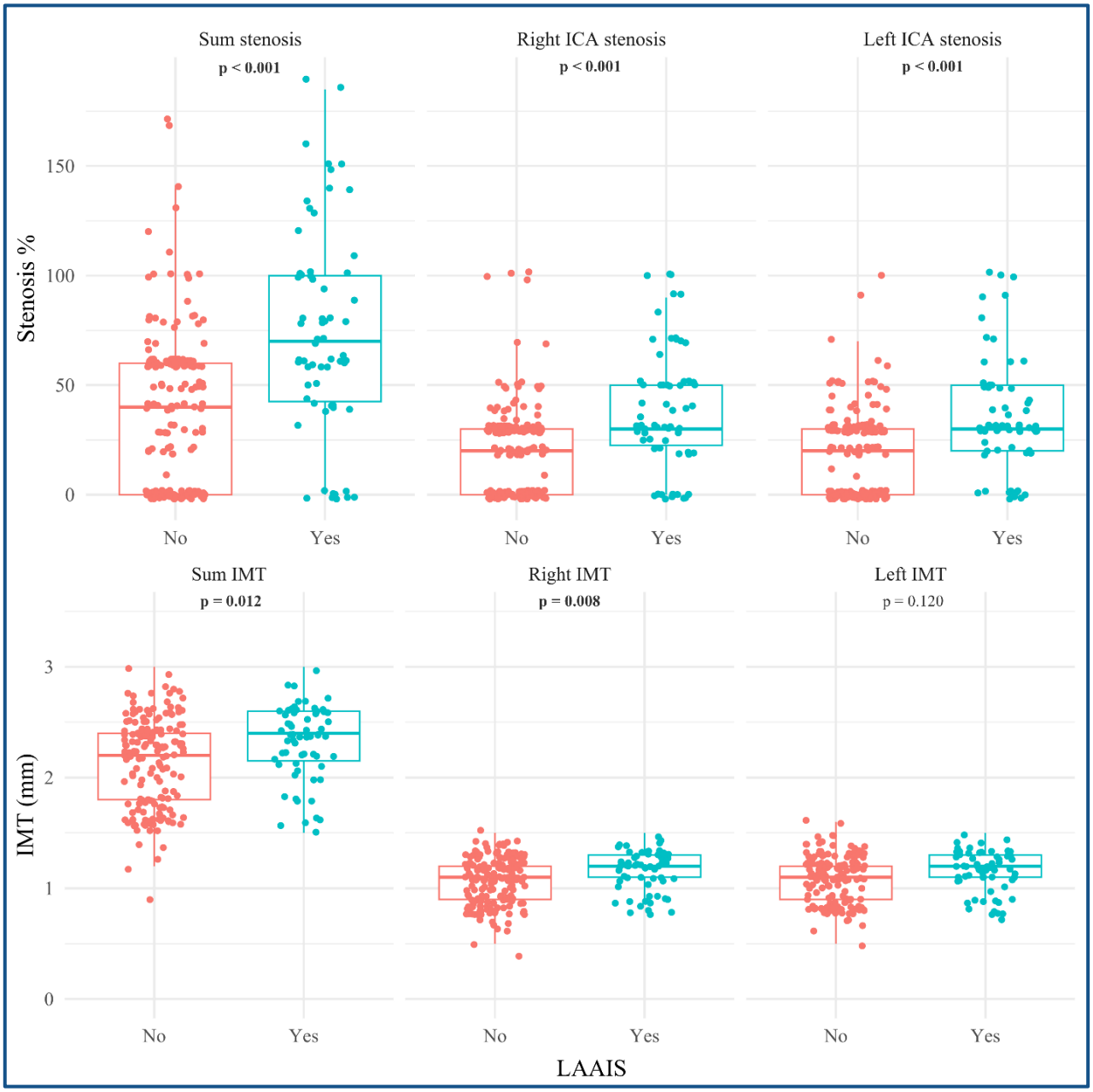

Figure 6. Differences in internal carotid artery atherosclerosis morphological parameters in patients with arteriolosclerotic CSVD with and without LAAIS, with p values. Abbreviations: ICA – internal carotid artery, IMT – intima-media thickness, LAAIS – large artery acute ischemic stroke.

From all neuroimaging markers of the disease, only WMH prevalence was different between groups, showing that patients with LAAIS had more WMH (54% vs 39%, p=0.044). However, there was no difference in WMH severity. The overall CSVD MRI burden score was also different between groups. CSVD patients without LAAIS had a greater prevalence of lower scores (scores 0 and 1), while in those with LAAIS, greater scores (scores 2 and 3) were more common (p<0.001) (Table 18, Figure 7). As with TOAST groups, NIHSS was also greater in those with LAAIS (p<0.001), and these patients had more atherosclerotic CVD (p=0.002) (Table 18).

Table 18. Differences in neuroimaging and clinical findings between arteriolosclerotic CSVD patients with and without large artery ischemic stroke

Characteristic	CSVD and LAAIS		
	No LAAIS, N = 178[1]	**Had LAAIS**, N = 63[1]	**p-value**[2]
WMH	70 (39)	34 (54)	**0.044**
CSVD MRI burden score			**<0.001**
score 0	15 (9.9)	2 (4.4)	
score 1	104 (69)	22 (49)	
score 2	30 (20)	14 (31)	
score 3	2 (1.3)	7 (16)	
NIHSS	5.00 (4.00 – 7.00)	7.50 (6.00 – 9.25)	**<0.001**
Atherosclerotic CVD	14 (7.9)	14 (22)	**0.002**

Abbreviations: CSVD – cerebral small vessel disease, LAAIS – large artery acute ischemic stroke, WMH – white matter hyperintensities, MRI – magnetic resonance imaging, NIHSS – National Institute of Health Stroke Scale, CVD – cardiovascular disease. Data adjusted for age.

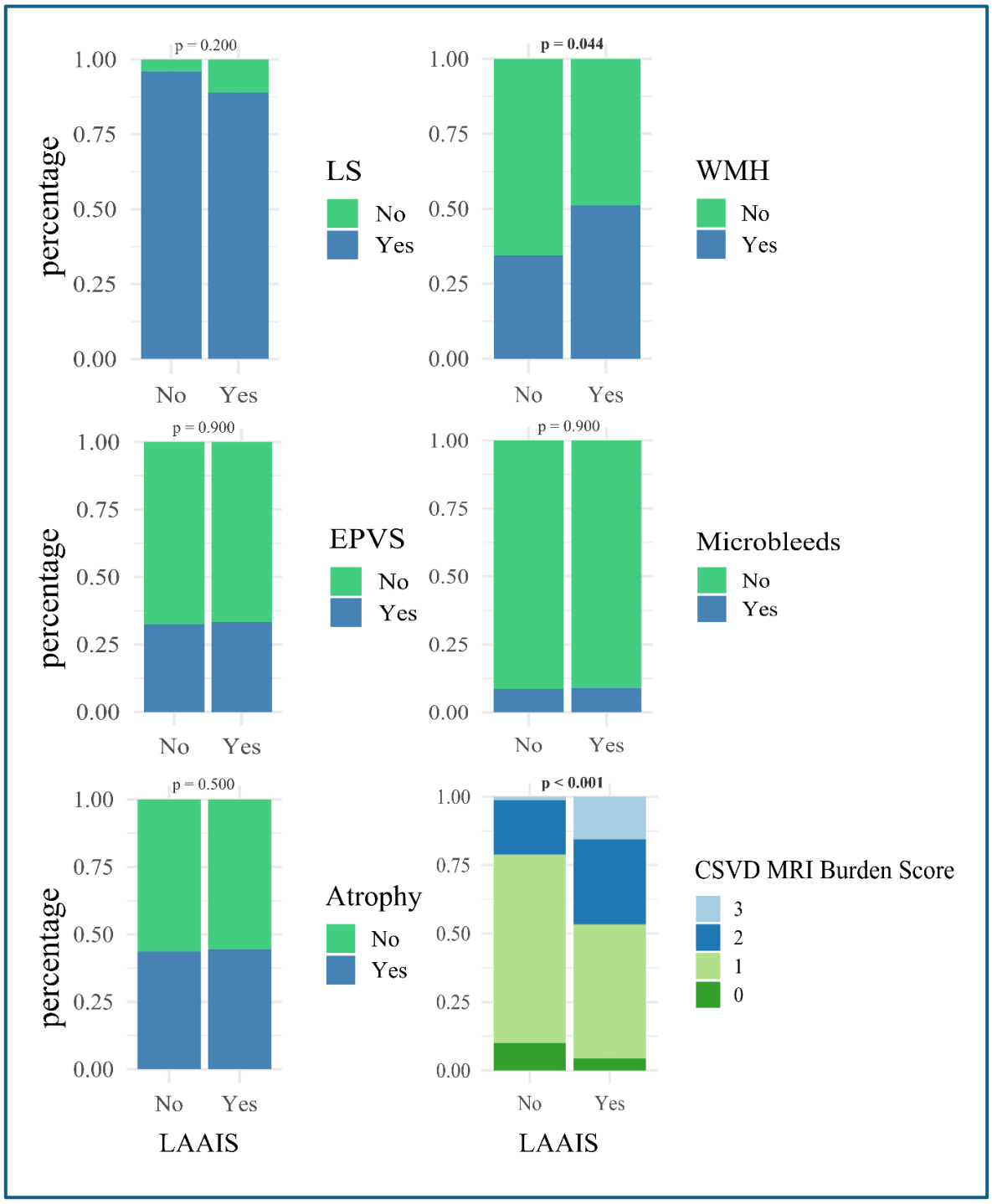

Figure 7. Differences in neuroimaging markers and the MRI burden score in patients with arteriolosclerotic CSVD with and without LAAIS. Abbreviations: LS – lacunar stroke, WMH – white matter hyperintensities, EPVS – enlarged perivascular spaces, CSVD – cerebral small vessel disease, MRI – magnetic resonance imaging, LAAIS – large artery acute ischemic stroke.

The total cholesterol in LAAIS patients was lower (p=0.002), and so was the HDL-C (p=0.006), but median triglycerides were higher than in patients without LAAIS (p=0.006). As for other laboratory findings, WBC, neutrophils, and NLR were higher in patients with LAAIS (p=0.002, p<0.001, p<0.001, respectively), with them also having a greater prevalence of increasing and elevated NLR (p<0.001). Folic acid levels were lower (p=0.030), but homocysteine levels were higher (p=0.048). Finally, on CSF analysis, patients with LAAIS had greater median levels of CSF albumin and the CSF to serum albumin ratio (p=0.046 and p=0.048, respectively). The detailed differences in laboratory findings are shown in Table 19 and Figure 8.

Table 19. Differences in laboratory findings between arteriolosclerotic CSVD patients with and without large artery ischemic stroke

	CSVD and LAAIS		
Characteristic	**No LAAIS**, N = 178[1]	**Had LAAIS**, N = 63[1]	**p-value**[2]
Total cholesterol	4.95 (4.26 – 5.82)	4.32 (3.78 – 5.10)	**0.002**
HDL-C	1.54 (1.17 – 1.97)	1.24 (1.10 – 1.54)	**0.006**
Triglycerides	1.19 (0.97 – 1.61)	1.36 (1.12 – 2.08)	**0.006**
WBC	6.90 (5.90 – 7.98)	7.50 (6.70 – 9.15)	**0.002**
Neutrophils	4.10 (3.40 – 5.00)	4.70 (3.98 – 6.50)	**<0.001**
NLR	2.21 (1.67 – 2.88)	3.01 (2.00 – 5.10)	**<0.001**
NLR groups			**<0.001**
low	0 (0)	1 (1.9)	
normal	70 (56)	13 (25)	
increasing	26 (21)	12 (23)	
elevated	30 (24)	26 (50)	
Folic acid	5.8 (4.6 – 8.6)	4.9 (3.2 – 6.1)	**0.030**
Homocysteine	12 (10 – 16)	14 (12 – 17)	**0.048**
CSF Albumin	0.45 (0.35 – 0.56)	0.59 (0.48 – 0.74)	**0.046**
CSF/serum albumin ratio	10.5 (8.8 – 13.8)	14.0 (11.3 – 17.6)	**0.048**

Abbreviations: CSVD – cerebral small vessel disease, LAAIS – large artery acute ischemic stroke, HDL-C – high-density lipoprotein cholesterol, WBC – white blood cells, NLR – neutrophil to lymphocyte ratio, CSF – cerebrospinal fluid. [1]n (%); Median (IQR). [2]Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test; Wilcoxon rank sum exact test. Data adjusted for age and sex (red blood cells, platelets, hemoglobin, creatinine, folic acid).

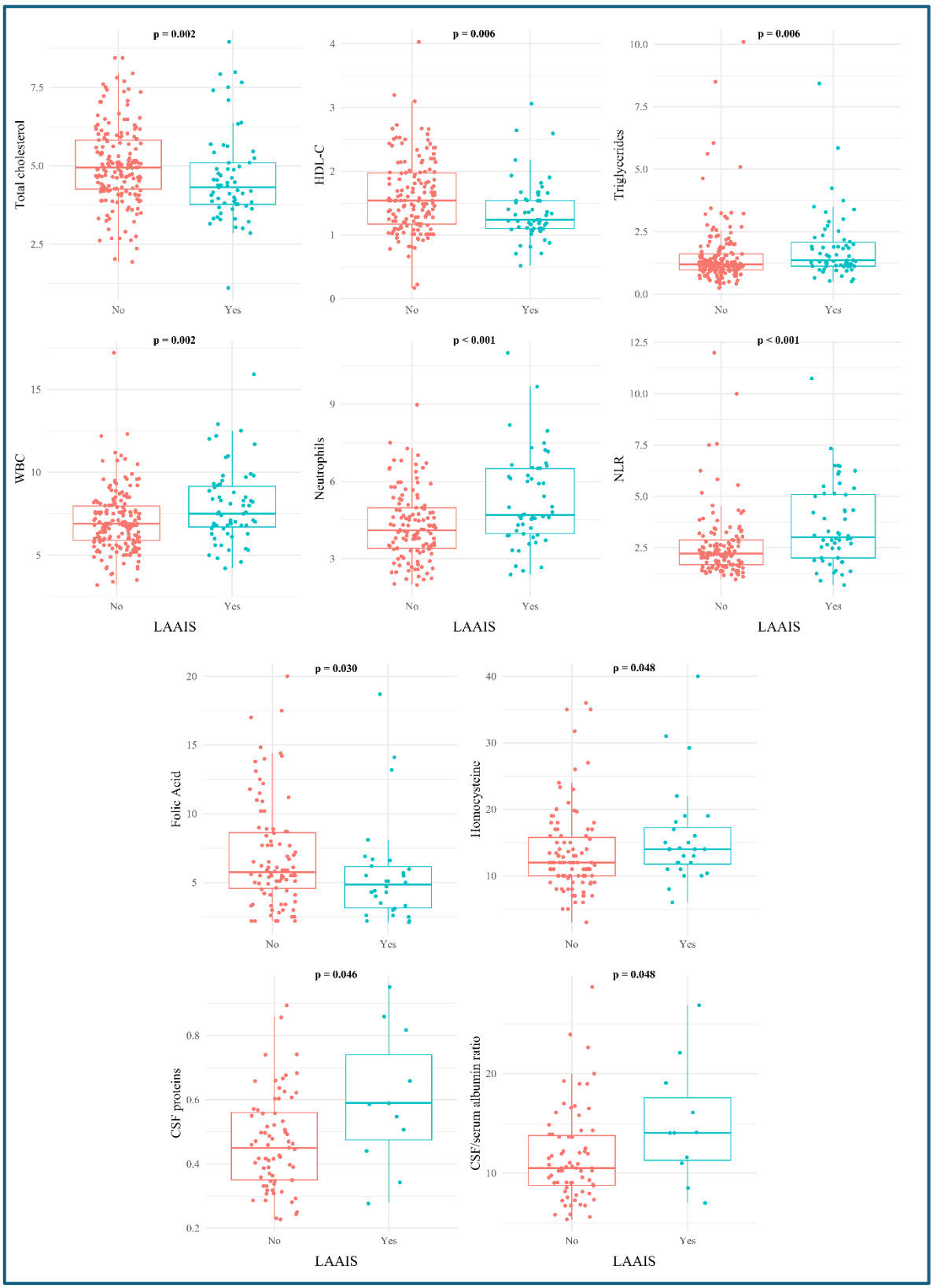

Figure 8. Differences in laboratory results in patients with arteriolosclerotic CSVD with and without LAAIS. Abbreviations: HDL-C – high-density lipoprotein cholesterol, WBC – white blood cells, NLR – neutrophil to lymphocyte ratio, CSF – cerebrospinal fluid, LAAIS – large artery acute ischemic stroke.

Next, we performed logistic regression analysis for each variable adjusting for age and sex, and calculated OR by exponentiating the regression coefficient. According to the results, patients with any ICA plaque had increased odds of LAAIS with an OR of 2.38 (95% CI 1.12-5.43, p=0.03). Those with anterior cerebral circulation stenosis on TCD also had greater odds of LAAIS (OR 5.47, 95% CI 1.28-25.5, p=0.023). Other important risk factors were the CSVD MRI burden score (OR 2.67, 95% CI 1.58-4.65, p<0.001), atherosclerotic CVD (OR 3.22, 95% CI 1.42-7.3, p=0.005), the NLR (OR 1.34, 95% CI 1.12-1.64, p=0.002), and the CSF to serum albumin ratio (OR 1.15, 95% CI 1.01-1.33, p=0.032). However, those with elevated total cholesterol and HDL-C had lower odds of stroke, with the OR for total cholesterol of 0.78 (95% CI 0.60-0.98, p=0.042) and the OR for HDL-C of 0.39 (95% CI 0.19-0.75, p=0.007). Detailed results for each variable are shown in Table 20 and Figure 9.

Table 20. Odds ratios for large artery stroke in patients with arteriolosclerotic CSVD, adjusted for age and sex

Characteristic	OR	95% CI		p-value
		low	high	
ICA plaque	2.38	1.12	5.43	**0.03**
Right ICA plaque	3.01	1.46	6.67	**0.004**
Right ICA stenosis %	1.03	1.02	1.05	**<0.001**
Right ICA stenosis degree	3.65	2.07	6.8	**<0.001**
Left ICA plaque	3.91	1.86	8.96	**<0.001**
Left ICA stenosis %	1.03	1.02	1.05	**<0.001**
Left ICA stenosis degree	3.78	2.12	7.16	**<0.001**
Sum stenosis	1.02	1.01	1.03	**<0.001**
Right IMT	10.7	2.11	60	**0.005**
Sum IMT	3.18	1.36	7.85	**0.009**
TCD anterior stenosis	5.47	1.28	25.5	**0.023**
CSVD MRI burden score	2.67	1.58	4.65	**<0.001**
Atherosclerotic CVD	3.22	1.42	7.3	**0.005**
Total Cholesterol	0.78	0.6	0.98	**0.042**
HDL-C	0.39	0.19	0.75	**0.007**
WBC	1.26	1.09	1.47	**0.002**
Neutrophils	1.55	1.24	1.97	**<0.001**
NLR	1.34	1.12	1.64	**0.002**
CSF Albumin	177	3.32	13 916	**0.013**
CSF/serum albumin ratio	1.15	1.01	1.33	**0.032**

Abbreviations: OR – odd ratio, CI – confidence interval, ICA – internal carotid artery, IMT – intima-media thickens, TCD – transcranial color Doppler, CSVD – cerebral small vessel disease, MRI – magnetic resonance imaging, CVD – cardiovascular disease, HDL-C – high-density lipoprotein cholesterol, WBC – white blood cells, NLR – neutrophil to lymphocyte ratio, CSF – cerebrospinal fluid.

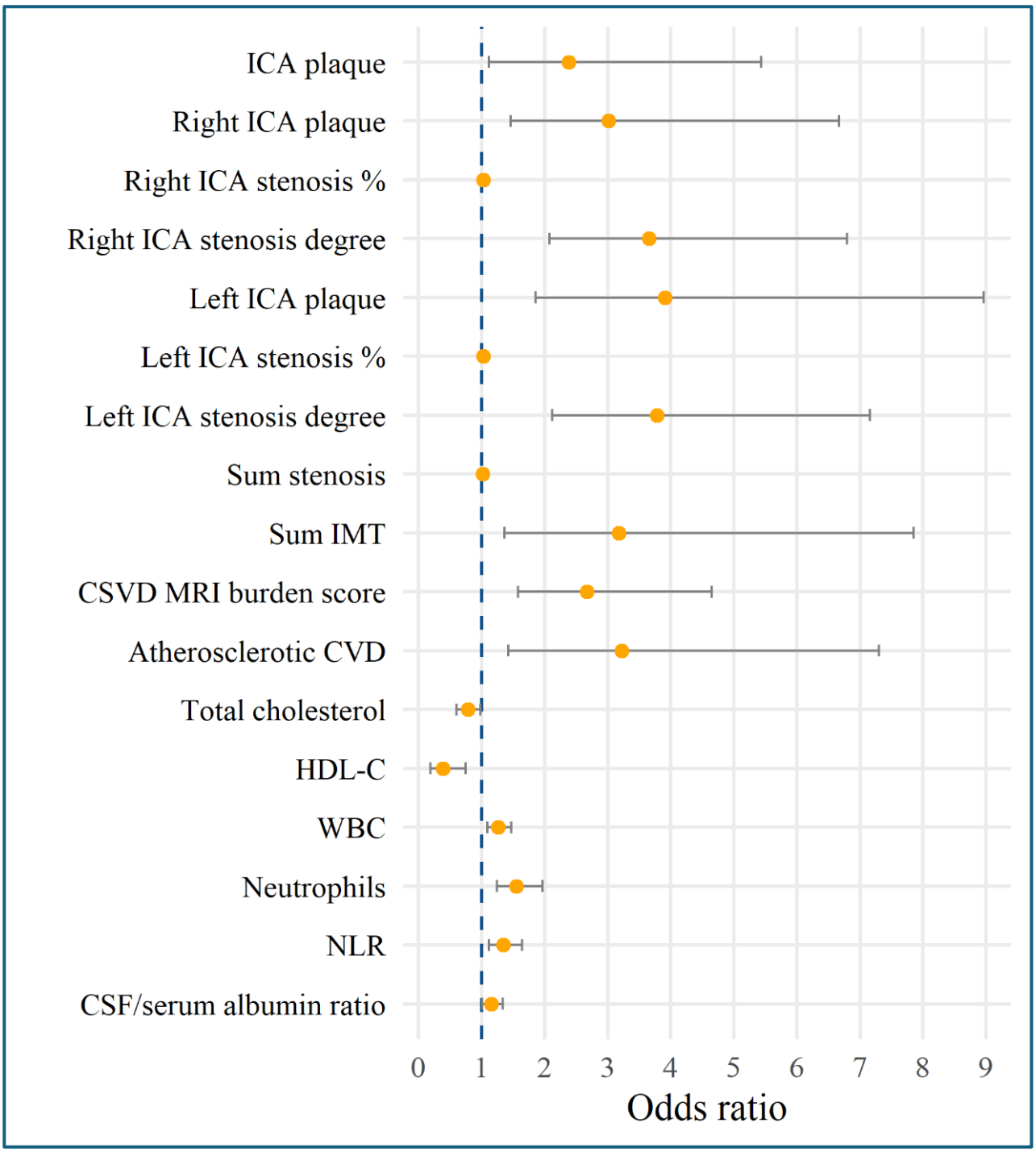

Figure 9. Odds ratios for LAAIS in patients with arteriolosclerotic CSVD, adjusted for age and sex. Abbreviations: ICA – internal carotid artery, IMT – intima-media thickens, CSVD – cerebral small vessel disease, MRI – magnetic resonance imaging, CVD – cardiovascular disease, HDL-C – high-density lipoprotein cholesterol, WBC – white blood cells, NLR – neutrophil to lymphocyte ratio, CSF – cerebrospinal fluid.

Finally, we calculated the RR for each risk factor for LAAIS in patients with arteriolosclerotic CSVD (Table 21). The presence of ICA plaques had an RR of 1.24 (95% CI 1.06-1.42), which significantly increased when looking at high-grade ICA stenosis only (RR 2.57, 95% CI 1.57-4.81). Having other atherosclerotic CVD also showed an increased RR of 1.54 (95% CI 1.13-2.37). As for "traditional" cerebrovascular risk factors, dyslipidemia showed an RR of 1.07 (95% CI 0.92-1.23), hypertension had an RR of 0.99 (95% CI 0.81-1.17), DM had an RR of 1.14 (95% CI 0.97-1.40), and

smoking had an RR of 0.94 (95% CI 0.80-1.11). "Non-traditional" risk factors had variable RRs for LAAIS. Decreased kidney function had an RR of 0.92 (95% CI 0.79-1.16) and systemic inflammation, characterized by the elevated NLR, had an RR of 1.49 (95% CI 1.19-1.98), while the BBB dysfunction, defined by the elevated CSF to serum albumin ratio, had an RR of 1.15 (95% CI 0.94-1.35).

Table 21. The relative risk for large artery stroke in patients with arteriolosclerotic CSVD

		95% CI	
Characteristic	RR	low	high
ICA atherosclerosis	1.24	1.06	1.42
ICA high-grade stenosis	2.57	1.57	4.81
Atherosclerotic CVD	1.54	1.13	2.37
Dyslipidemia	1.07	0.92	1.23
HTN	0.99	0.81	1.17
DM	1.14	0.97	1.40
Smoking	0.94	0.80	1.11
Decreased kidney function	0.92	0.79	1.16
Systemic inflammation (elevated NLR)	1.49	1.19	1.98
BBB dysfunction (elevated CSF/serum albumin ratio)	1.15	0.94	1.35

Abbreviations: RR – relative risk, CI – confidence interval, CVD – cardiovascular disease, ICA – internal carotid artery, HTN – hypertension, NLR – neutrophil to lymphocyte ratio.

5. DISCUSSION

The median age of patients included in our study was 65, with an IQR of 56-73. There were slightly more females in our cohort than males (54% vs 46%). In other studies on CSVD, which are not specifically geared toward a certain age or gender, ages usually range around 65 years. When it comes to sex, some studies have more females, while others have more males, but no study has a substantial difference in sex in their cohort, which is similar to our population (33,42,98,102,103,127). The prevalence of hypertension was high at 78%, which is common for type 1 arteriolosclerotic CSVD, as this type is even referred to as hypertensive CSVD (1,5). Other well-known cerebrovascular risk factors (dyslipidemia, DM, smoking) were prevalent in our cohort. Out of all, DM had the lowest prevalence at 30%. Even though DM is traditionally considered a risk factor for CSVD, recent studies have challenged that notion. The work of Inkeri and associates showed that glycemic control in patients with type 1 diabetes wasn't associated with asymptomatic CSVD, which was present in our cohort (40). On the other hand, the prevalence of smoking was very high in our cohort, even higher than in other studies on CSVD. However, considering that our population sample is from Serbia, a country with a high prevalence of smoking, this is not surprising (48,50,158).

On imaging, LS were the most common finding. This is expected, as LS are correlated with hypertension, smoking, and diabetes, all of which were prevalent in this population (5,159). Additionally, LS are common in arteriolosclerotic CSVD, possibly due to their pathology being directly related to arteriolosclerosis and atheroma. In a study by Hua et al., which dealt with arteriolosclerotic CSVD patients, the prevalence of LS was 98% (160). In our cohort, WMH were present in 43% of all patients, and EPVS in 33%. Microbleeds had the lowest prevalence at 8.7%. This distribution of CSVD imaging markers is expected in arteriolosclerotic CSVD. As mentioned before, WMH are associated with hypertension, EPVS with inflammation, while microbleeds are the least prevalent CSVD marker in arteriolosclerotic CSVD (5,87). The burden score in our cohort ranged from 0, in patients with only symptomatic LS, to 3. Only 9 (4.6%) patients had a score of 3, but no patient had a score of 4. This is similar to other studies, as a severe CSVD burden is not a common finding (54,104).

When assessing differences between males and females in our cohort, we showed that male patients had higher median sum stenosis, right, left, and sum IMT. This finding has been previously shown by studies that specifically looked for differences in ICA atherosclerosis between sexes. One systematic review including 42 studies on ICA plaques and their characteristics reported that plaques in men were more commonly larger and were more likely to have calcifications, intraplaque hemorrhage, and ulcerations (161). As there weren't many patients with high-grade ICA stenosis in our cohort, we didn't expect to show these findings. However, the difference in sum stenosis between males and females indicates worsening ICA atherosclerosis in men. IMT was previously shown to be larger in men. Stevens et al. measured IMT in patients between 45 and 65 years of age at baseline and 3 times at 3-year intervals. Each time, IMT in both right and left common carotid arteries was greater in men (162). Interestingly, more recent studies have shown differences in IMT values between men and women, even in younger individuals around 20 years of age. Mazurek et al. showed that IMT values were higher in men even after adjusting for vascular risk factors. On the other hand, even though Cromwell et al. didn't find differences in IMT between young men and women, their findings indicate worse cardiovascular health in young adult males (163,164).

Out of all CSVD imaging markers, the presence of microbleeds and the number of EPVS showed differences between men and women. Microbleeds were more prevalent in men. The population-based Rotterdam study included 3979 individuals and had a goal of assessing the prevalence and risk factors for microbleeds. The authors found that there is no difference between men and women in the prevalence of microbleeds in all age groups. They did associate smoking with

microbleeds, which could be a factor in our cohort, as men were more commonly smokers than women. It should be noted that the Rotterdam study included healthy individuals and not CSVD patients. There is also a geographic difference between our own cohort and the Rotterdam study that should be taken into account (13). Another important study, the Framingham Heart Study, included 1965 healthy individuals. The mean patient age was 65, and the study included 54% female patients, making their cohort similar to our own. Interestingly, the authors found that microbleeds were present in 8.8% of patients, which is similar to 8.7% in our cohort, and that microbleeds were more common in men. They also attributed low total cholesterol to the presence of microbleeds. Even though we didn't assess the differences in each imaging factor separately, in our study, men had lower total cholesterol, which could be correlated to microbleeds (165). Lu et al. analyzed the UK Biobank, including 8159 participants, and found that microbleeds were detected in 7% of patients. Men had a larger prevalence of lobar microbleeds, although we wouldn't be able to comment on this finding as we did not differentiate microbleeds based on the cerebral territory (166). Lastly, Fandler-Höfler et al. performed a pooled analysis of 38 prospective studies including 20314 patients with stroke across different geographic locations. They found that microbleeds were more common in men in all age groups, independent of geographic location, racial, or ethnic background. The difference between sexes was specifically pronounced in those over the age of 60. The same authors found that LS were more common in men, while WMH were more common in women (167). We didn't have any statistically significant findings regarding LS and WMH between men and women, possibly due to our study dealing with arteriolosclerotic CSVD with high prevalence of WMH and LS, making it difficult to attribute these findings to one sex. There is also a possibility that a larger cohort would reveal this finding. When it comes to EPVS, sex is not considered a risk factor, and most authors didn't find an association between the presence of EPVS and sex (168–170). Ramirez et al. assessed the number of EPVS in elderly patients and found that men had a greater number of EPVS, particularly in the white matter. Even though our study evaluated the number of EPVS in the basal ganglia, it shows the same trend (171). Choe et al. evaluated the association between EPVS and cognition in patients from a memory clinic with a wide range of cognitive dysfunction, which is a population closer to our own. The authors found that male sex was associated with a higher degree of EPVS in the basal ganglia (172).

Even though the difference in ABCD2 scores between men and women is statistically significant, the median for both groups is the same, with a slight difference in IQR (men: 4 IQR 4-6, women: 4 IQR 3-5). Purroy and associates evaluated sex differences in clinical features of TIA and found that there are no differences in the ABCD2 score between sexes, but did note that atypical TIA events are more common in women. It is possible that the discrepancy in our study comes from the fact that we didn't include or quantify atypical TIA events, or that patients didn't report these events in their histories (173).

There was a difference in smoking, with more smokers being men. This finding is expected for the population of Serbia, as the prevalence of smoking is higher in men than in women (158). There were no statistically significant differences in other risk factors, but the analysis did reveal that women in our cohort had higher levels of total cholesterol and HDL-C. This fact can be attributed to normal cholesterol findings in the general population (174). However, the association between lipids and CSVD in men and women separately has been previously described. Yin et al. found that they were inversely associated with CSVD lesions on MRI in women but not in men. Of note, in the elderly, which is our population, all lipid levels decrease, but this decrease is more pronounced in men (175), which could be a factor in our study.

Our analysis revealed differences in RBC and hemoglobin levels between male and female patients, with female patients having lower values for both. This is a well-known physiological difference, and normal reference ranges for these labs are different for men and women (176). Interestingly, men had a higher prevalence of low hemoglobin, indicating anemia. This is a surprising finding, as anemia is usually more common in women (177). The association between anemia and CSVD is shaky at best. While some authors show no correlation between anemia and CSVD, there is

a correlation with cortical atrophy, cognitive impairment, and dementia, which is one of the ways CSVD presents (178). Others point to the correlation between anemia and separate imaging features of CSVD, like WMH (179,180). There is another important correlation regarding anemia, which could play a role in our cohort, and that is to the development of microbleeds. Tan et al. evaluated the association of low hemoglobin with CSVD imaging markers and cognition in 796 individuals ages 60 and older. Besides the previously described association with cortical atrophy and cognitive impairment, authors describe the association between low hemoglobin and microbleeds (181). This is an interesting finding for our cohort as men had a higher prevalence of microbleeds that we previously discussed. As for the platelets, their levels were higher in women. Platelet count is dependent on sex and decreases with age, but after the age of 15, women have a higher count than men (182,183).

Urea, creatinine, and kidney dysfunction also showed differences between sexes. In fact, men had higher levels of urea and creatinine, and a higher prevalence of kidney dysfunction. Urea levels are dependent on the glomerular filtration rate, so the increased values in men are to be expected with an increase in the prevalence of kidney dysfunction. Higher levels of urea in men have been shown previously by other authors (184). As for creatinine, kidney dysfunction could be a contributor, but there is also a physiological difference between men and women, attributing higher levels of creatinine in men to the fact that men typically have more muscle mass than women (185). Kidney dysfunction itself is considered a possible risk factor for CSVD (63,64). As kidneys represent another way of visualizing small vessel function, kidney dysfunction could be related to worse cardiovascular health in men in our population, shown by the increased markers of ICA atherosclerosis, microbleeds, greater EPVS load, low HDL-C, and smoking status.

The next difference is seen in folic acid, with women having higher levels, which is an expected difference between the sexes (186). This finding could also be coupled with anemia being more prevalent in men in our study, or women taking folic acid supplements, but we didn't evaluate for these factors as that was not the aim of this study. Even though higher homocysteine levels in men could be attributed to expected sex differences as well, in our cohort, there was a significantly higher prevalence of hyperhomocysteinemia in men (186). As mentioned, hyperhomocysteinemia has been associated with CSVD and disease burden (69,70). However, we didn't find any differences in disease burden between men and women. We believe that hyperhomocysteinemia in men could be a marker of their cardiovascular health and ICA atherosclerosis. One systematic review evaluated 19 studies dealing with homocysteine levels and subclinical markers of atherosclerosis, including IMT. They found that homocysteine was consistently higher in men and that in most studies, it correlated with markers of atherosclerosis after the adjustment for age, sex, and risk factors. As lowering homocysteine didn't lead to IMT improvement, the authors concluded that homocysteine could serve as a marker of atherosclerosis. This is still a debated topic, as some authors show that the correlation between CSVD and homocysteine exists only in women (187). In our study, men showed greater atherosclerosis and were more commonly smokers, which could be associated with their increased homocysteine level and point to worse cardiovascular health than women (188).

The final difference between sexes is evident in the BBB dysfunction shown through increased CSF albumin and CSF/serum albumin ratio, as well as a higher prevalence of elevated CSF/serum albumin ratio in men. This finding has been replicated in previous studies in neuropsychiatric patients, as well as individuals from the general population (189–191). In the work of Parrado-Fernández et al., the authors evaluated the CSF/serum albumin ratio in over 20000 anonymous patients and found consistently elevated values in men in all age groups when compared to women (190). Meixensberger et al. found the same difference between male and female patients with schizophreniform and affective psychosis. The authors attempted to elaborate on the mechanism and attributed it to the difference in height between men and women. As men are generally taller than women, their spinal column is also taller, meaning that there is a longer distance between the site of the CSF production (ventricles) and the site where the sample is obtained (lumbar spine). In theory, there is an increasing gradient of CSF/serum albumin ratio, where values are higher further away

from the site of CSF production (ventricles), leading to elevated values in men (191). Other authors attribute the difference in the ratio to the function of the BBB. In vitro studies have shown that women have lower BBB permeability and increased BBB strength. Additionally, estrogen increases nitric oxide production, leading to vasodilation and increased cerebral blood flow, which can accommodate the metabolic requirements of the brain (192). Based on our findings, we decided that variables in which the influence of sex is clear (i.e., red blood cells, platelets, hemoglobin, creatinine, folic acid) should be adjusted for sex as a confounding variable. As it is not clear if CSF/serum albumin ratio sex differences are physiological or pathological, nor is it suggested that this ratio requires adjustment for sex, we decided to use values adjusted for age only, as with other variables.

The statistical analysis revealed a difference in age among CSVD MRI burden score groups, meaning that patients with higher scores were older. This finding is reasonable, as aging is considered a non-modifiable risk factor for CSVD (7,8). Additionally, other authors previously described the correlation between the CSVD MRI burden score and age (9,10). Hilal et al. studied the prevalence of CSVD in 1797 individuals. Even though the authors didn't measure the overall CSVD MRI burden score, they found that each separate imaging marker of CSVD and their severity increased with age, indicating increased disease burden in older patients (9). Additionally, Fan et al. studied the incidence of silent CSVD in young adults and found that age is directly correlated with the prevalence of silent CSVD in these patients, with each separate marker increasing in prevalence with age (10). It would stand to reason that if each marker increases in prevalence with age, the overall burden will also increase, as there is a higher likelihood that older patients would have multiple CSVD imaging markers overlapping at the same time. Lu et al. focused on the correlation between carotid artery stenosis and the CSVD MRI burden score but noted an additional finding, which involves aging and disease burden. In their cohort, patients with higher disease burden were older than those with lower burden (104).

The big difference among CSVD MRI burden score groups is seen in morphological markers of ICA atherosclerosis. In fact, in our cohort, all morphological markers of ICA atherosclerosis (presence of plaque, stenosis degree, and IMT) on ultrasonography have shown increased values with higher CSVD MRI burden scores. Other authors who studied this correlation had similar results to our own. For example, Del Brutto et al. studied the correlation between atherosclerosis and CSVD burden in Atahualpa individuals over the age of 60. Of note, the included individuals didn't have a diagnosis of CSVD, hence the burden score in this cohort was somewhat lower than in our own. However, the authors concluded that there is a strong correlation between carotid artery stenosis and the CSVD MRI burden score, which was especially evident in patients with a higher disease burden (54). Similar findings were described by Lu et al. Even though their cohort had more male patients (74%), they found an association between the CSVD burden and carotid artery stenosis (104). When looking at high-grade ICA stenosis only, Fan et al. didn't find an association between stenosis degree and the CSVD burden score. However, they did show that intraplaque hemorrhage is associated with an increased CSVD burden. These findings would be difficult to replicate in our cohort. Our cohort is made up of arteriolosclerotic CSVD patients, while Fan et al. included patients who had high-grade ICA stenosis, regardless of CSVD diagnosis. On that note, our cohort included mostly patients with low-grade stenosis. We didn't evaluate for the presence of intraplaque hemorrhage, as its associated finding on ultrasound, ulceration, wasn't present in any of our patients. Additionally, in Fan's cohort, CSVD burden ranged from 0-4, with 6.5% having a score of 3, and 7.4% having a score of 4, which are higher than in our population. Even though the authors didn't find a clear correlation, they argued in favor of using the CSVD burden score as a marker due to its correlation with clinical symptoms (33). As our patients mostly had low-grade asymptomatic stenosis, it is difficult to make a conclusion whether this correlation is causal or not. Low-grade asymptomatic ICA stenosis is often overlooked both clinically and in research. One study with a cohort of patients with low-grade stenosis, the SMART-MR study, showed an association of low-grade carotid stenosis with the progression of brain atrophy and cognitive decline, which are features of CSVD, over a period of 12 years (84). Authors attributed these findings to low-grade ICA stenosis causing decreased brain perfusion and hypoxia,

leading to cognitive decline. In our study, we didn't find a correlation between ICA plaque hemodynamic parameters and CSVD burden score, which indicates that brain perfusion is maintained in our cohort. In addition, other authors found that atherosclerosis in different vascular beds is also correlated with CSVD burden. Johansen et al. describe the association between coronary artery stenosis and WMH, while Song et al. found that patients with aortic atheromas had a worse CSVD burden (85,193). With this in mind, we would theorize that ICA plaques are not directly correlated with the CSVD burden but serve as a window to the pathology of small brain vessels.

Vasomotor reactivity showed a correlation with the burden score, but only on the left side. This finding can be pathologically linked to CSVD because vasomotor reactivity serves as a measurement of cerebral small vessel autoregulation. In CSVD, stiffening of the vessel wall due to arteriolosclerosis impairs the vessels' ability to dilate and autoregulate in response to increased oxygen needs (26). A recent case-control study by Liu et al. confirmed this pathology in CSVD, showing that cerebral autoregulation was impaired in CSVD patients and independently correlated with imaging findings (194). However, even with these findings being logically attributed to CSVD pathology, we wouldn't be able to speculate on their correlation or validity, due to the small number of patients for this specific analysis. In total, 33 patients had both VMR and CSVD MRI imaging burden quantified, out of which only 4 had low VMR, which is not enough to make any valid conclusions. A larger cohort would be needed to confirm this correlation.

All CSVD imaging markers, independently, showed an increasing prevalence and severity with an elevated burden score. This correlation is purely methodological, as CSVD imaging features are used to calculate the overall burden score, and the burden score serves as a reflection of imaging features and their severity (98). The only imaging feature not included in the overall burden score is brain atrophy. In our study, there was a correlation between the prevalence of brain atrophy and the CSVD MRI burden score, showing that atrophy prevalence increases with the burden. Even though it is not a component of the burden score, atrophy is a marker of the disease (5,6,98). Atrophy not only progresses over time but is also associated with severe disease and cognitive impairment in CSVD patients. Fan et al. showed that CSVD burden correlates with medial temporal lobe atrophy. This finding could be attributed to our analysis, but it should be taken into account that we didn't define atrophy of separate brain regions, but brain atrophy in general. A study by Staals et al. that evaluated CSVD burden in patients with LS and mild LAAIS found that both deep and superficial atrophy were associated with the CSVD burden on imaging (50). Of note, there are some other factors with a correlation to the burden score in our study that have been previously implicated in brain atrophy. ICA atherosclerosis, aging, hypertension, and homocysteine levels have been previously linked with brain atrophy and could act as confounders in this analysis (71,71,84).

The next statistically significant findings refer to the prevalence and severity of LAAIS. Patients with higher CSVD MRI burden scores had a greater prevalence of LAAIS. Stroke in these patients was also more severe, as evidenced by increasing NIHSS. Stroke and other cardiovascular diseases have been previously correlated with CSVD burden. Xu et al. evaluated the correlation between CSVD burden and a composite of major adverse cardiac and cerebrovascular events (MACCE), including stroke, myocardial infarction, revascularization procedures, and all-cause mortality. They found that increased CSVD burden predicts poor prognosis, indicated by MACCE (195). Similar findings were described by Goldstein and associates, correlating CSVD burden with all-cause mortality (196). In a recent study, Pinheiro et al. evaluated the association between the CSVD burden score and the risk of stroke in the Framingham Heart Study participants. The authors found that the CSVD burden score and risk of stroke were associated in a dose-dependent manner, meaning the higher the burden score, the higher the hazard ratio (HR) was (197). This finding is very interesting for our study, as the Framingham Heart Study and our cohort share similarities previously described (in age, sex, and imaging marker prevalence), making results comparable. A prospective study using the Rotterdam cohort followed up neurologically healthy patients for 7 years for the occurrence of stroke, dementia, and all-cause mortality, and measured CSVD MRI burden score. After the prospective period, the authors showed the association between all three outcomes (stroke,

dementia, death) and a higher disease burden. Some important differences between this cohort and our own should be pointed out. The Rotterdam cohort included neurologically healthy patients, while ours included CSVD patients treated at the Neurology clinic. Additionally, there is a difference in age (the mean age of the Rotterdam cohort is 73.3 years) (198). However, even with these differences, the findings from both Framingham and Rotterdam cohorts show that the CSVD burden increased the risk of stroke even if the disease itself is asymptomatic, which is very common with CSVD (27). The correlation between CSVD and LAAIS could stem from the pathological mechanism of CSVD. As there is vascular damage in CSVD and decreased autoregulation of small blood vessels, the vessels can't adapt to increasing oxygen needs, making the brain tissue more prone to ischemic events (33,199). This association is even more clear if we take into account that increased CSVD burden has been associated with poor prognosis after stroke, worsening recovery, higher risk of cerebral bleeding after treatment, and increased stroke recurrence (200–205). The correlation between the CSVD burden and stroke is so important that the American Heart Association suggests interventions for primary stroke prevention in patients with asymptomatic CSVD defined by its imaging markers. In this instance, the CSVD MRI burden score could serve as an important tool for patient stratification (198,206).

The next statistically significant finding, hypertension is expected for this condition. Not only is type 1 arteriolosclerotic CSVD referred to as hypertensive CSVD, but hypertension has been linked with separate imaging findings like WMH and EPVS, both of which are prevalent in arteriolosclerotic CSVD. (1,5) Klarenbeek et al. in their study described the influence of blood pressure on CSVD burden, showing a positive correlation between the two (98). More recent studies correlate blood pressure fluctuations to the occurrence of CSVD and its burden. In the aforementioned study by Xu et al., the authors found that abnormalities in blood pressure variability are strongly associated with CSVD burden (195). One meta-analysis including 19 studies on blood pressure variability and CSVD found that variabilities in either systolic or diastolic blood pressure were correlated with the CSVD burden and could be used as predictors for disease progression. There are some inconsistencies between the included studies, with some giving more value to systolic, while others considered diastolic blood pressure as having a higher impact on the CSVD burden (207). In our study, we didn't specify systolic and diastolic blood pressure or blood pressure variability as this wasn't the main goal of the study. However, our finding of a higher prevalence of hypertension with an elevated CSVD burden does point to the correlation between the two, as shown by other authors. Even with a large number of studies showing this correlation, the pathologic mechanism behind it is still unclear. Some authors theorize that it is not the elevated blood pressure but fluctuations in blood pressure that cause vascular injury and endothelial dysfunction, which lead to brain ischemia (207,208).

Systemic inflammation marked by elevated WBC and neutrophils, as well as NLR, has been correlated with the CSVD burden score. In fact, all 3 markers have shown an increase with a higher burden. Since lymphocyte values didn't correlate with the burden score, we can conclude that the WBC increase comes from neutrophils themselves. As mentioned before, NLR has been investigated in various conditions and found to be associated with aging, cardiovascular disease, and cancers. Even though there are several studies correlating NLR to AIS, the ones focusing on CSVD are lacking. In CSVD specifically, NLR has been associated with cognitive decline and disease burden (60–62,209). In a case-control study by Wang et al. NLR correlated with CSVD, but also with the CSVD burden score and the outcome (62). While Hou et al. showed an association between NLR and cognitive impairment, which is often seen in CSVD, we wouldn't be able to compare these results as we didn't assess cognitive function in our patients (61). One study assessed the effect of multiple inflammatory markers on the CSVD burden. The authors didn't find an association between NLR and the CSVD burden but did describe a strong association between neutrophils and the burden score. Even though this cohort is similar to our own in its characteristics, like age and sex, it is important to point out that the authors used a population of community-dwelling individuals, not specifically CSVD patients, which is why NLR values were lower in this cohort and more similar to healthy individuals. NLR in patients who had imaging markers of CSVD was around 1.74. In contrast, the aforementioned work

by Hou et al. had an NLR median of 2.59 in patients with cognitive impairment and 2.21 in those without, and Wang et al. showed a median NLR in CSVD patients of 1.97, and 1.51 in the control group, which are closer to our median NLR value (2.38) (61,62,100). Lastly, Cai et al. specifically assessed the differences in NLR between CSVD patients and controls. They found that NLR was associated with separate imaging markers of CSVD and their severity. Also, NLR showed the highest sensitivity and specificity in predicting CSVD (209). Even though the neutrophil count is correlated with the CSVD imaging burden in our study and in the work of Hou and associates, we believe that NLR presents a better marker of inflammation for CSVD patients. In the acute setting (i.e., AIS), neutrophils infiltrate the brain tissue, causing the generation of reactive oxygen free radicals, promoting the release of inflammatory mediators, and triggering systemic inflammation. Their levels become rapidly elevated in the first few days, significantly drop around day 7, and return to baseline values (in the brain) in a month or up to a few months (210). This dynamic makes the evaluation of the involvement of neutrophils as predictors difficult, which is why NLR could serve as a better marker as it evaluates the relationship of neutrophils to lymphocytes, allowing to sort-of normalize their levels. On the other hand, in CSVD, usually, there is no acute event (except for symptomatic LS), hence the inflammation is chronic and low-level. In atherosclerosis, neutrophils worsen the damage and increase endothelial dysfunction and vessel permeability, disrupting the BBB (211,212). Therefore, neutrophil levels are relatively constant and could be used when assessing patients without acute symptoms.

In our analysis, we showed that urea is increased in patients with a high CSVD burden when compared to those with a lower burden. As we discussed, kidney function has been previously correlated with CSVD, but with variable results. While Yao et al. describe the correlation between the progressive kidney disease and the exacerbation of WMH and microbleeds, another study on the Framingham cohort revealed an association of kidney function with CSVD itself, but not separate CSVD markers (64,213). The varying results of the association between kidney function and CSVD have been recognized by a meta-analysis that pooled results from 53 studies. They found that eGFR had a null effect on CSVD, while there was a correlation between categorical kidney function and CSVD, especially with WMH (214). Tanaka et al. showed a strong correlation between eGFR values and albuminuria independently with the CSVD MRI burden score in patients on antithrombotic therapy for stroke prevention. Most patients in their cohort had stage 2 kidney disease marked by eGFR values between 60 and 90 mL/min/1.73m2 (215). However, in our study, the glomerular filtration rate didn't correlate with the CSVD MRI burden score. There are some factors to consider here. For our analysis, lab values were obtained from the hospital information system. The hospital laboratory reports only eGFR below 60 mL/min/1.73m2 as decreased. In chronic kidney disease, stages 1 and 2 involve eGFR between 60 and 100 mL/min/1.73m2 (216). Due to this way of reporting, we were able to identify only patients with moderate and severe kidney dysfunction, so most of the patients in our cohort are considered to have normal kidney function. In that sense, there could be a subgroup of patients with mildly decreased kidney function, in which case, urea levels could serve as a substitute marker. The relationship between urea and CSVD has been studied by Nam and associates. The authors used urea to albumin ratio as a parameter of kidney and endothelial dysfunction and attempted to correlate it with the volume of WMH and the presence of LS and microbleeds in 3012 seemingly healthy individuals. The results revealed an association between the urea-to-albumin ratio and WMH volume and LS separately (217). The authors attributed this correlation to the vascular similarities between the brain and kidneys. They also noted another possibility in relation to urea itself. Sympathetic activity is implicated in CSVD (218). However, when there is increased sympathetic activity, kidney excretion is decreased, and urea levels rise. Therefore, urea levels could indicate increased sympathetic activity in CSVD patients and identify those who are at risk of disease progression (217).

When assessing the effect of homocysteine, we didn't find a correlation between homocysteine levels and the CSVD MRI burden score. Of note, this association was present in the unadjusted data but was lost after adjusting for other factors. This was an unexpected finding, as other

studies have shown this correlation. For example, Cao et al. in a Mendelian randomization study showed a correlation between homocysteine levels and the CSVD burden. This study included community-dwelling individuals, regardless of their diagnosis, in which case there is a possibility of including different subtypes of CSVD, while our cohort included only arteriolosclerotic CSVD. Additionally, mean homocysteine levels were at 18 μmol/L, significantly higher than in our cohort (70). In a study by Teng and associates, the authors evaluated the correlation between homocysteine, CSVD imaging burden, and cognition in patients from the memory clinic, excluding patients with acute symptoms. Homocysteine values were log-transformed, so we couldn't compare these results with our findings. However, this cohort had a significantly higher prevalence of microbleeds and a higher CSVD MRI burden, which could indicate a mixed etiology of CSVD, particularly the involvement of CAA, which is also associated with Alzheimer's disease and dementia (71). CAA has been previously associated with high homocysteine levels and a high MRI burden (219,220). Studies on the involvement of homocysteine in separate subtypes of CSVD are lacking. One meta-analysis including 18 studies found that there is a significant heterogeneity in studies assessing the correlation between homocysteine and CSVD. The authors categorized findings based on imaging and found the difference between homocysteine levels was higher in patients with WMH vs controls than LS vs controls (69). This could be a factor in our cohort as LS were a lot more prevalent than WMH. In the SMART-MR study, authors correlated hyperhomocysteinemia with WMH volume and the presence of LI, and worse cognitive function (221). Even though we didn't find an association between homocysteine levels and the CSVD MRI burden score, we did discover that the prevalence of hyperhomocysteinemia increases with the disease burden with borderline statistical significance ($p=0.05$). This finding could point to the involvement of homocysteine, but probably not in a dose-dependent manner.

The final statistically significant result in this part of the analysis shows that fT3 levels are the highest in patients with an MRI burden score of 3, followed by the score 0 group. This finding could be attributed to previous studies associating thyroid hormones, especially elevated fT4 with white matter lesions in CSVD (78). However, there is a discrepancy in findings among studies, as other authors correlated markers of hypothyroidism (fT4) with cognitive impairment and depression, which are both clinical features of CSVD (77,79). Additionally, both high and low fT4 levels were linked with reduced brain perfusion. Authors of this study attribute their findings to chronic elevations of fT4 being associated with hypertension and atherosclerosis, which affect brain perfusion, while explaining that hypothyroidism can lead to decreased cardiac output and even heart failure, which also affect cerebral perfusion (222,223). There are a few issues when commenting on the findings of our analysis regarding fT3. First, there is a lack of studies on the fT3 and CSVD. To our knowledge, some studies correlate low fT3 values and poor outcomes after AIS (224–226). Second, the mechanism behind fT3 involvement in brain health is not well understood. Free T3 can cross the BBB and might be involved in promoting neural development as well as neuroprotection in ischemia (227). Third, the findings of different studies on the involvement of thyroid function in CSVD are variable, as discussed before. Finally, our own findings should be considered carefully. The median fT3 went from 4.53 with a score of 0 ($n=13$), to 4.01 with a score of 1 ($n=69$), 4.01 with a score of 2 ($n=21$), and 7.01 with a score of 3 ($n=2$). As there are only 2 patients in the score 3 group with fT3 findings, these results could be influenced by the sample size, so there is a possibility that the correlation is inversed in reality and that fT3 actually becomes lower with an increasing CSVD burden, indicating subclinical hypothyroidism.

When looking at the differences between symptomatic LS (TOAST type 3) and LAAIS (TOAST type 1), we identified the different degrees of ICA stenosis. In our study, all morphological markers of ICA stenosis showed greater values in patients with LAAIS when compared to those with LS. ICA stenosis is a common cause of LAAIS and a well-known risk factor for LAAIS (228). Tejada et al. in their study compared the presence of ipsilateral and contralateral high-grade ICA stenosis in patients with LS vs those with LAAIS. Results showed that higher ICA diameter stenosis was more common in patients with LAAIS, which is in accordance with our findings. The authors also showed

that ICA stenosis on the ipsilateral artery to the LS was more common than ICA stenosis in the contralateral artery, indicating the role of ICA stenosis in LS. (105) This notion is somewhat controversial as other authors have different opinions on the topic. In the systematic review by Jackson et al., the authors included 41 studies on various risk factors in stroke subtypes. When looking at ICA stenosis, they found that both ipsilateral and contralateral stenosis had a stronger association with non-lacunar strokes (130). Additionally, Inzitari and associates described that LS patients more commonly had milder stenosis than LAAIS patients. However, carotid endarterectomy for high-grade ICA stenosis reduced the risk of stroke in all patients, but to a lesser degree in those with LS (229). Therefore, the association between ICA stenosis and LS is not clear.

Another finding related to cerebral circulation is the increased pulsatility index in the intracranial section of the right vertebral artery in patients with LAAIS. PI is a measure of vessel elasticity and vascular resistance (230). Arterial pulsatility is highly associated with CSVD and CSVD-related lesions (231). Due to the pathological stiffening of cerebral vessels in CSVD, there is a decrease in the elastic recoil, which could translate to increased vascular resistance and PI of large arteries (26,230). However, this theorizing is speculative in our situation as we didn't find differences in hemodynamic parameters of other intracranial large arteries.

The next statistically significant finding involved the CSVD MRI burden score, with LAAIS patients having a higher prevalence of severe CSVD burden. This is somewhat of an expected finding that relates to the pathology of CSVD. CSVD changes in the brain lead to a hostile environment and impaired autoregulation, which is why these patients are more prone to having an AIS (232). In a prospective study by Staszewski et al. CSVD patients and the control group (without CSVD but with a high risk of atherothrombosis) were followed up for 2 years and evaluated for vascular events (hemorrhagic stroke, ischemic stroke, and TIA) and death. CSVD patients had about 4 times the risk of vascular events or death than the control. The risk was not dependent on the symptoms of CSVD (i.e., acute or chronic) but did increase if patients had a higher disease burden on MRI (233). One limitation of this analysis should be noted. Due to the methodology of calculating the CSVD MRI burden score, patients with symptomatic LS would have a lower score by default as a point is granted only for asymptomatic LS. In practice, they wouldn't be able to score a maximum possible score of 4 (98). This would also explain the discrepancy between our findings and the findings from Staals et al., who described a higher CSVD burden with LS than LAAIS. However, when calculating the CSVD MRI burden, the authors used a scoring system in which 1 point is awarded for any LS (symptomatic and asymptomatic). In that sense, if we were to adapt their scoring to our own, we would get the same findings of LAAIS having a higher CSVD burden (50).

The finding of lower NIHSS in LS patients is not novel. Aldriweesh and associates studied the clinical characteristics of stroke subtypes defined by the TOAST criteria in 989 stroke patients. They described lower NIHSS at admission in patients with LS (mean of 5) in comparison with LAAIS patients (mean of 8) (234). Moreover, Arba et al. found an NIHSS median of 6 in LS patients and also suggested that an NIHSS of 7 or less could increase the specificity of identifying LS (89). In this sense, our finding isn't novel but serves as a confirmatory finding attributing to data validity.

Regarding risk factors, there was a difference in the prevalence of atherosclerotic CVD, DM, and total cholesterol, but not the prevalence of dyslipidemia, hypertension, or smoking. These cerebrovascular risk factors are a common finding in both LS and LAAIS. The presence of atherosclerotic CVD in stroke subtypes has been researched before. The findings from Aldriweesh et al. show that ischemic heart disease (the most common finding in our atherosclerotic CVD variable) was more commonly present in patients with LAAIS (234). Staals et al. found the same trend, but it wasn't statistically significant, which could be attributed to the sample size. Additionally, authors separated PAD and myocardial infarction, while in our cohort, those two were combined into one variable (50). Both LS and LAAIS are atherosclerotic manifestations, just like myocardial infarction and PAD. As LS affects one penetrating arteriole, and LAAIS affects large arteries, it would require advanced atherosclerosis to cause LAAIS, which is not necessarily the case with LS. This coincides

with our finding of ICA plaque morphologic parameters being more advanced in LAAIS. Lv et al. performed an analysis of risk factors among stroke patients, grouping their cohort based on the stroke subtype. They discovered that patients with LS were older and had a higher prevalence of DM, smoking, and hypertension. There are some differences between this study and our own that could explain the contradictory results. In this cohort, patients with any AIS were included, while our cohort included CSVD patients, some of whom had LAAIS. As CSVD is considered an age-related disease, we wouldn't expect to find any differences between groups here. The overall prevalences of smoking and hypertension were high in our cohort, so it would be unexpected to be able to associate those findings with the stroke subtype in our study specifically. There is also an influence of the population as our study included the Caucasian population, while Lv. et al. performed their analysis on the Asian population (235). Interestingly, one meta-analysis compared the association of risk factors and AIS subtypes in Asian and Caucasian populations and found that hypertension was not associated with LAAIS in Caucasians, while this association existed in the Asian population (236). Jackson et al. also described the influence of hypertension among multiple studies, accounting for the possibility of bias. They found that hypertension was associated with LS more commonly in studies that favored LS diagnosis based on the presence of risk factors. The authors who defined ischemic stroke without considering risk factors had only a slight association between hypertension and LS (pooled RR of 1.11) (130). As for the association with DM, Lv et al. showed that the prevalence of DM is lower in patients with LAAIS when compared to LS. Our analysis had the opposite finding, with LAAIS having a higher prevalence of DM (235). This could be attributed to the pathological mechanism of the disease. Again, it should be kept in mind that our cohort was exclusively made up of CSVD patients. As we previously mentioned, pathological changes in the CSVD lead to a hostile environment and impaired autoregulation, putting these patients at risk for AIS (232). As diabetes affects small vessels and is involved in CSVD pathology, it would be reasonable to assume that DM would be more often present in patients with disease complications (i.e., LAAIS). Our finding of lower total cholesterol in LAAIS patients seems a bit odd at first. However, this finding was described before. In the previously mentioned study by Inzitari et al. comparing LAAIS and LS, the authors determined that dyslipidemia was an important risk factor for LS, even more than hypertension (229). The work by Jackson sums up the correlation clearly. When looking at total cholesterol, the pooled RR for LS was 1.22, based on which the authors suggested that patients with elevated cholesterol have a stronger predisposition for LS than other stroke subtypes (130). It should also be considered that patients with LAAIS had a greater prevalence of atherosclerotic CVD, and some of them already had a diagnosis of CSVD prior to LAAIS, so there is a possibility that they could've already started taking lipid-lowering medications, which would influence these findings.

When it comes to inflammation, we discovered a difference in the neutrophil count and the NLR between groups. Both markers were elevated in patients with LAAIS when compared to LS. As discussed before, neutrophils are involved in ischemic brain injury, which occurs in both LS and LAAIS (210). As LS are smaller and less severe lesions of the two, it would be reasonable to assume that there would be less involvement of inflammatory cells (i.e., neutrophils). Most of the studies assessing the NLR focus on AIS in the general population or CSVD, without evaluating subtypes of stroke or imaging markers of CSVD separately. In relation to stroke, Tokgoz has shown that NLR levels at admission might have predictive value for short-term mortality. The authors also measured NLR values for stroke subtypes and reported that in LS, the NLR median was 3.2, while in LAAIS, it was 6.5, which shows the same trend as our results. These values are somewhat higher than what we described. We believe this discrepancy comes from the point in time when CBC was obtained. While Tokgoz et al. measured NLR at admission, when neutrophils reach their peak, our measurement was performed at a later time point to avoid the influence of the acute event (237). Studies looking at CSVD imaging markers assessed the correlation of the NLR to lacunes specifically, which are long-term consequences of LS. There are some variable results here. Cai et al. showed no correlation between the NLR and lacunes after adjusting for confounding factors like age, sex, and risk factors. These findings were replicated in separate studies by Nam et al (209,238). This would fit with the pathological mechanism of lacunes. LS are areas of active ischemia that over time become lacunes

after undergoing volume reduction (1,6). Fisher described lacunes as areas of liquefactive necrosis that, in chronic stages, appear like cavities with fine connective tissue and fatty macrophages that diminish in numbers over time (19,22). As neutrophils and the NLR are markers of inflammation, their activity seems to illustrate this mechanism.

Hypercoagulation and the involvement of coagulation factors have been implicated in both LS and LAAIS (239,240). The findings of studies on the topic are variable, with some indicating an increased factor VII activity in stroke and LS, and others pointing to factor VII deficiency-related thrombosis as a potential cause (241,242). Additionally, some authors discovered that factor VII polymorphism is associated with stroke, while others show that there is no correlation between the two (243,244). One genome-wide association study identified new loci for factor VII and found that increased activity of this factor is associated with stroke. Even though these are interesting findings, we wouldn't be able to relate this to our study as the authors didn't use stroke subtypes, and due to a different methodology used (245). We would report this finding as a possibility but wouldn't attempt to make any assumptions or conclusions based on it as there is a lack of evidence to support this correlation.

The main findings of this study involve the differences between CSVD patients with and without LAAIS. LAAIS patients had a higher degree of ICA stenosis based on all morphological parameters on extracranial ultrasonography. As mentioned before, the correlation here is clear, as ICA stenosis is a common cause of stroke. About 34% of all ischemic strokes are due to thromboembolism originating from the ICA. Importantly, about one-third of these patients will have asymptomatic stenosis over 50% (246). Additionally, CSVD affects cerebral circulation and the environment, increasing the odds of stroke and worsening its prognosis (232,247). As CSVD affects the vascular reserve of the brain and with it vascular autoregulation, when carotid artery stenosis causes decreased perfusion pressure, small vessels aren't able to dilate, leading to hypoxia, which promotes ischemic events (199,248). The involvement of large artery stenosis in correlation with CSVD has been repeatedly found and recognized (1,33,84,105–107). The risk of stroke in CSVD patients has been established, but hasn't been thoroughly researched in the setting of carotid artery stenosis (6). Han et al. studied the association between large artery stenosis, CSVD, and AIS in 1082 patients, whom they followed up for 6 years. The authors found that the high-grade ICA stenosis (HR = 3.27) and the CSVD imaging burden (HR = 12.73) were associated with an increased risk of stroke (34). Moreover, Dai and associates evaluated the relationship between high-grade ICA stenosis and the CSVD burden in LAAIS patients and found that stroke patients with severe ICA stenosis or occlusion had a higher CSVD burden (249). These findings further highlight the correlation between the 3 conditions (CSVD, LAAIS, ICA stenosis) and the influence of an altered brain environment and reduced vascular reserve on the increased occurrence of LAAIS, even if it originates from ICA stenosis.

Regarding intracranial circulation, the increased prevalence of large artery intracranial stenosis in the anterior circulation indicates an advanced atherosclerotic burden in these patients. Intracranial artery stenosis has previously been correlated with CSVD, and it was shown that they share the same risk factors (250,251). Zhu et al. studied the correlation between the CSVD imaging burden and intracranial plaques and found that the CSVD imaging burden and separate imaging markers (LS and WMH) had a positive correlation with intracranial artery stenosis. The authors additionally described the correlation between unilateral vulnerable plaques and the ipsilateral CSVD imaging burden (252). When looking at CSVD and intracranial stenosis in the setting of stroke, Chen et al. found that only patients with intracranial stenosis had an increased risk of stroke, but patients who had both CSVD and intracranial stenosis had more neurological disability after the stroke (253). Lastly, Li et al. studied the influence of CSVD with intracranial stenosis on the occurrence of AIS and found that patients who had AIS more frequently had both CSVD and intracranial artery stenosis (254). One limitation of this finding should be pointed out. Most of the patients in our cohort had LAAIS involving MCA, meaning in the anterior circulation. The finding of intracranial stenosis on TCD in the anterior circulation could be related to MCA, leading to stroke, in which case, there would be a methodological explanation for the finding. As this is a cross-sectional study in which variables

were measured at only one time point, we wouldn't be able to tell if anterior circulation stenosis on TCD occurred before or after the LAAIS. Our finding of decreased VMR as an indicative marker of altered cerebral autoregulation has been elaborated on in the previous section.

Neuroimaging markers of CSVD are indicators of disease burden and progression. WMH specifically are associated with an increased risk of stroke across multiple studies, with some authors attributing WMH to stroke recurrence and severity (255–260). In a meta-analysis by Debette et al., the authors included 6 population-based studies and 6 studies in populations at high risk for AIS (i.e., those with atherosclerosis). They found that the pooled HR in population studies was 3.1, while in high-risk population studies, it was 7.4, making the overall HR 3.5. This could be attributed to the shared vascular risk factors for WMH and stroke (age and hypertension). However, even after adjusting for risk factors, all included studies showed that the association between WMH and AIS remained statistically significant (261). In a more recent review and meta-analysis focusing on all imaging markers of CSVD, Debette et al. showed that WMH were again associated with an increased risk of AIS (262). These findings, coupled with our own results showing that out of all CSVD imaging markers, only WMH were more prevalent in LAAIS patients, indicate that WMH carry a heavier burden on brain microvasculature than other CSVD lesions. WMH are usually described to occur from reduced cerebral blood flow that leads to BBB breakdown and demyelination, but recent studies have linked neuroinflammation as a mechanism of WMH formation (1,5,263). These mechanisms, combined with the fact that WMH are found in the watershed areas of the brain, show that WMH might be a sign of a global issue in the brain vasculature, which is why these patients are more prone to AIS. A higher CSVD burden in LAAIS patients has been shown in the previous analysis and elaborated on the mechanism of CSVD lesions affecting brain circulation, making patients more prone to LAAIS. As for the NIHSS, in CSVD patients, this score stems exclusively from those with symptomatic LS. This correlation has been shown and elaborated previously on the basis of LS being less severe than LAAIS (89,234).

Out of all risk factors, only the presence of atherosclerotic CVD was different among groups and more prevalent in CSVD patients with LAAIS. Most of the patients with atherosclerotic CVD in our cohort had cardiac ischemia. The correlation between LAAIS and myocardial infarction is well-known, and patients with one condition are at an increased risk of the other (264). This correlation is expected as both stroke and myocardial infarction share the same vascular risk factors found in arteriolosclerotic CSVD patients (5,265). Even though dyslipidemia itself didn't correlate with the occurrence of LAAIS, these patients had lower levels of total cholesterol and HDL-C, but higher levels of triglycerides. This could partially be explained by the previously described finding from Jackson et al. that patients with higher levels of total cholesterol are more likely to have LS rather than LAAIS (130). The association between cholesterol and LAAIS is traditionally considered to be positive, as authors usually describe that elevated total cholesterol carries an increased risk of AIS (266). When subtyping the type of AIS, findings show that increased total cholesterol seemingly favors smaller and less severe strokes. Elevated total cholesterol has been correlated to LS, and some authors point out that cholesterol plays a greater role in the development of CSVD than LAAIS (267–269). As for the HDL-C, its association with AIS is considered to be negative. Sacco et al. described that HDL-C had a protective effect in regard to ischemic stroke, especially in the case of atherosclerotic stroke (270). These findings were shown by other authors as well (271–273). When it comes to triglycerides, other authors have shown their association with both AIS and CSVD. In a study by Varbo et al., the authors found that increased triglycerides were associated with an increased risk of AIS, but in the case of cholesterol, only severely increased values were associated with an increased risk of AIS (274). When thinking about lipids in relation to stroke and CSVD, it is not enough just to consider their levels, but the interplay of the lipid metabolism. Lipoproteins rich in triglycerides include very low-density lipoproteins and chylomicrons. These lipoproteins get converted into cholesterol-ester-enriched remnant particles during lipolysis. While the very low-density lipoproteins and chylomicrons cannot cross the endothelium due to their size, remnant particles can. Therefore, they can penetrate the arterial wall and have atherogenic properties (275).

The finding of elevated WBC, neutrophils, and NLR could be attributed to the inflammation. NLR in association with CSVD and has been described previously. We will summarize the findings that pertain to NLR in CSVD patients and LAAIS here. As NLR is indicative of inflammation, which is long-standing and chronic in CSVD, it would show that CSVD patients with higher inflammatory activity are more likely to have LAAIS, probably due to inflammation reflecting damage to the brain vasculature (61,100,133,209).

Elevated levels of homocysteine have been described as a risk factor for CVD since the 1960s, which is confirmed in our cohort. Since then, hyperhomocysteinemia has been correlated with CSVD, cognitive impairment, and LAAIS through the mechanism of oxidative stress, neuronal apoptosis, and depletion of B12 from glial cells (69–72,276). We believe that there is an interplay between homocysteine levels and folic acid levels in our cohort, thereby we would suggest that low baseline folic acid levels in LAAIS patients are probably associated with elevated homocysteine. There is a possibility of low folate intake in these patients, but we didn't perform this assessment, so we wouldn't be able to account for it. Folic acid supplementation has been studied in stroke and CSVD with variable results. Cavaleri et al. studied the effect of folate and vitamin B12 and B6 supplementation in patients with recent stroke or TIA after 2 years. The authors found that supplementation had no effect on reducing the progression of CSVD lesions, but suggest that there could be an effect on WMH, which they might have missed due to a small number of patients having severe CSVD burden (75). There is an idea of using folic acid supplementation earlier in the disease progression. Such case-control study was performed by Vermeulen et al. on a cohort of patients with atherosclerotic disease (plaques in extracranial or intracranial cerebral arteries). The authors found that patients who received folic acid showed decreased progression of atherosclerosis (277). There have been several studies on the relationship between folic acid deficiency and AIS. Weng et al. showed that folate status was correlated with the incidence of ischemic stroke. Weikert et al. studied this effect on 25770 individuals ages 35 to 65 and found that B12 deficiency and combined deficiency in B12 and folic acid increased the risk of AIS and TIA. This differs a bit from our findings as we didn't associate B12 levels with LAAIS. The discrepancy between the findings could come from the fact that we didn't account if any patients were taking B12 supplementation. Also, our cohort is older than what the authors used for their study, and folic acid levels are known to decrease with age, so we probably had less variability (278–280). Van Guelpen and associates had different findings. Their study included 334 ischemic and 62 hemorrhagic stroke patients. Study results showed that folic acid is protective for hemorrhagic but not for ischemic stroke (281). We wouldn't be able to comment on these findings as one of the exclusion criteria in our cohort was hemorrhagic stroke.

The role of BBB dysfunction in stroke has been established, but only in situations where AIS was the cause of the dysfunction. In ischemic stroke, there is an obstruction of blood flow causing ischemia, which induces oxidative stress and neuroinflammation, leading to BBB dysfunction. Moreover, BBB dysfunction causes increased permeability of the BBB, which might increase stroke area, lead to hemorrhagic transformation or poststroke epilepsy, and has been associated with poor prognosis (282,283). To our knowledge, there are no studies assessing BBB dysfunction as a risk factor for stroke. Xu et al. in their review describe BBB dysfunction as one of the key pathologies in chronic cerebral hypoperfusion, which is also present in CSVD due to altered autoregulation and decreased vascular reserve (199,248,284). There is a vast number of studies assessing the BBB dysfunction in dementia, showing that it is present in just about any type of dementia and that it can be detected before the onset of dementia, allowing BBB dysfunction to serve as a biomarker (58,285–288). As the CSF/serum albumin ratio is a measurement of the BBB integrity and correlates with the CSVD burden, it could represent another measurement of disease progression and therefore be used to identify patients who are at an increased risk of stroke. One key aspect of our methodology shouldn't be ignored. In the majority of our patients, CSF analysis was performed during their first hospital stay. Even though we attempted to use the findings that are further away from the acute episode, there is a possibility that the BBB dysfunction identified with the CSF/serum albumin ratio

in LAAIS patients stems from their AIS. Therefore, these findings would have to be replicated in a prospective study with multiple measurements.

The regression analysis, OR, and RR calculations revealed some important findings as well. Even though there is a large number of studies assessing risk factors for stroke or CSVD, studies evaluating risk factors for stroke in CSVD patients are lacking, making it difficult for us to compare and assess the validity of our findings. All morphological markers of ICA stenosis were more severe in LAAIS patients, and almost all showed a correlation on logistic regression analysis and OR values over 1 (Table 20, Figure 9). When looking at asymptomatic ICA stenosis, the Asymptomatic Carotid Stenosis and Risk of Stroke Study is the largest study on the topic. This study included 1121 patients with asymptomatic high-grade stenosis who were followed up for 48 months on average. The cumulative 5-year stroke rate was 4%, 7%, and 12% for mild (50-69%), moderate (70-89%), and severe (90-99%) ICA stenosis, respectively. Based on these findings, the authors concluded that the stenosis severity alone was not a good indicator of stroke risk (289–291). In a meta-analysis by Howard et al. ICA stenosis showed a linear correlation with AIS, with an OR of 2.1 in moderate-to-severe stenosis. Interestingly, when comparing high-grade to low-grade asymptomatic ICA stenosis, OR for stroke was 1.4 in the 50-69% stenosis, 2.3 in the 70-79% stenosis, and 3.2 in the 80-99% stenosis group. Based on these findings, the authors concluded that even though the risk of stroke due to asymptomatic ICA stenosis declined over the years, it still remains high in patients with high-grade stenosis, which could be an indicator of underestimation of the benefits of surgical intervention (292). In our cohort, the ORs for right and left high-grade ICA stenosis were 3.65 and 3.78, significantly higher than reported in population-based studies. We also showed an elevated RR for the presence of ICA plaques and high-grade stenosis of any ICA.

Intracranial artery stenosis carries a great risk of stroke, contributing to about 8-10% of all ischemic events (293). In the Barcelona-Asymptomatic Intracranial Atherosclerosis study that involved 933 individuals with vascular risk factors but no history of ischemic events, the authors found an increased risk of stroke (HR=1.83) in the case when asymptomatic intracranial stenosis was present (294). Another study using the Oxford Vascular Study cohort that included 92728 individuals, evaluated the risk of recurrent stroke in patients with minor AIS and TIA. Intracranial vessels were assessed using MR or CT angiography, or alternatively, TCD. In this study, patients with no intracranial stenosis were compared to those with 50-99% intracranial stenosis. Based on the Cox regression, it was found that there is an increased risk of recurrent stroke in patients with symptomatic intracranial stenosis (HR = 1.43) (295). Even though these studies don't include CSVD patients, but are population-based, their findings could be related to our own. While these 2 large studies show that both intracranial asymptomatic and symptomatic stenosis carry a risk of stroke and recurrent stroke, our findings relate this risk in the setting of CSVD, showing a high OR and elevated risk of LAAIS in these patients.

In our cohort, patients with an increased CSVD MRI burden score had higher odds of LAAIS. This is somewhat similar to findings from other authors. In the population-based Rotterdam study, the authors found that HR for stroke was 1.54, while in the previously described Framingham cohort, HR for a score of 1 was 1.73, for a score of 2 it was 1.83, and those with a score of 3 or more had an HR of 3.31 (197,198). It should be noted that both Rotterdam and Framingham cohorts are population-based including neurologically healthy individuals. Some authors didn't assess the overall burden score, but the association of separate CSVD imaging markers with LAAIS. Fu et al. and Imaizumi et al., in their separate studies, looked for a correlation between WMH grade and stroke. They found that WMH carried a high risk for recurrent stroke, with Imaizumi noting that recurrent strokes presented as LS or intracerebral hemorrhage (296,297). Both of these studies point to the devastating effect of CSVD on brain circulation and its contribution to poor stroke outcomes. In our study, even though WMH were more frequent in LAAIS patients, logistic regression didn't reveal an influence on the stroke occurrence. As this was a cross-sectional study, we didn't perform any assessment for stroke recurrence. Microbleeds were researched by Fan et al. and Haji et al. in their separate studies. Both studies involved patients who had AIS and were evaluated for stroke

recurrence. Fan noted that microbleeds were associated with intracerebral hemorrhage, while Haji reported a negative finding (298,299). In our cohort, we had a very low prevalence of microbleeds, probably due to them being rare in arteriolosclerotic CSVD, therefore, we couldn't make any correlation with stroke (5,9).

When looking at the risk factors for stroke, the one that was identified in our study is the existence of other atherosclerotic CVD. AIS has been considered the most feared complication of myocardial infarction (300,301). Coronary syndrome and AIS share common risk factors, so the coexistence of the two is not surprising (5,265). Sobiczewski et al. studied the correlation between symptomatic CAD and AIS in a cohort of 1183 patients who underwent diagnostic coronary angiography. The patients were followed up for about 7 years, in which time 50 of them developed a stroke, 43 being AIS. Interestingly, most patients had LS, while LAAIS was the second in prevalence. In the COX hazard regression mode, CAD involving multiple vessels had an HR of 1.8. Unfortunately, the authors didn't perform a regression analysis for each stroke subtype, so it is not possible to know what HR for LAAIS specifically is (302). In our analysis, we didn't assess the correlation between atherosclerotic CVD and LS, so we wouldn't be able to speculate on this matter. Additionally, most of our patients had LS, so this analysis wouldn't reflect the actual influence of CVD on AIS subtypes. In another study on the Rotterdam cohort, IMT and aortic calcifications were identified as the strongest risk factors for AIS (303). When discussing atherosclerosis in correlation with AIS, PAD is also considered a risk factor. In a review by Banerjee et al. pooled OR for stroke or TIA in patients with low ankle-brachial index, which is used to assess for PAD, was 2.33 (304). These findings and our own indicate the importance of atherosclerotic CVD and atherosclerosis itself on the occurrence of stroke in CSVD patients.

Both total cholesterol and HDL-C have shown an OR of less than 1, meaning that they have an inverse relationship to AIS. We would be careful when interpreting these results. As previously described, elevated total cholesterol favors CSVD development rather than large artery disease (267–269). Therefore, we wouldn't call this a protective effect. As for the HDL-C, it was described as having a protective effect against LAAIS (270–273). In the aforementioned study by Sacco et al. HDL-C had an OR of 0.53, showing that these patients have lower chances of AIS (270). Zhang et al. had similar results, showing the OR for AIS in the high HDL-C group of 0.71 (271).

We report NLR as a strong predictor of LAAIS in CSVD patients, with an elevated OR and among the highest RR out of all risk factors, second to high-grade ICA stenosis. Similar findings were described by Saliba et al. when assessing the NLR as a prognostic marker for AIS in patients with atrial fibrillation. In this population-based study that included 77297 adults with atrial fibrillation, the authors found that AIS incidence was the highest among patients in the 4th quartile for NLR. Moreover, the COX regression model revealed that adding the NLR to the CHA2DS2VASC score improved the ability to identify patients at risk of AIS. The authors suggested that the connection comes from the fact that inflammation is involved in atrial fibrillation, similarly to how in our cohort, CSVD is an inflammatory condition (305). When looking at neurologically healthy adults, Suh et al. followed up 24708 individuals ages 30-75 for about 6 years. The authors found that the risk of stroke increased with the baseline NLR values in a dose-dependent manner (NLR < 1.5 had an HR of 1.76, while the NLR ≥ 3.5 had an HR of 2.96) (306).

Even though the CSF/serum albumin ratio has been repeatedly reported as a marker for the progression of neurological diseases including dementia, there is a lack of studies to support its use as a biomarker for the risk of AIS. This possibly stems from the invasiveness of the CSF collection and the fact that it is collected during hospitalization, so the chances of having this analysis performed before the stroke are very low. In our study, we reported an increased OR and RR with the elevation of this marker. However, the limitation of its collection during the patients' hospital stay has been discussed. Our last set of findings relates to RR of vascular risk factors. We showed that hypertension, smoking, dyslipidemia, and decreased kidney function had an RR close to 1, while the RR of DM

was slightly elevated. These findings indicate that "traditional" cerebrovascular risk factors are shared between large and small vessel disease and are not usable as predictors of LAAIS in CSVD patients.

The main strength of this study comes from the cohort used. As CSVD is an umbrella term encompassing different etiologies affecting the same vessels, we considered it important to try and create a cohort including only 1 type of CSVD, which would help focus on this specific etiology and avoid genetic and other influences. Our study included patients with type 1 arteriolosclerotic CSVD, which was confirmed by the demographic factors similar to other studies on the topic (age and sex), the presence or risk factors related to this condition (hypertension), and the lack of imaging markers that are not associated with this condition (microbleeds). Due to these elements, we believe that our study is generalizable to a larger population of patients with type 1 arteriolosclerotic CSVD. This study also involved the evaluation of various risk factors that are already clinically available or could be obtained with minor calculations. This fact makes our study easily translatable into clinical practice. The final strength of our study can also be considered as its limitation. We set out to assess the influence of risk factors in CSVD patients predisposing them to LAAIS. This very specific aim is a strength regarding the use of its findings and avoidance of confounding factors. However, this is also a limitation, as there is a lack of studies with this specific aim, making it difficult for us to compare and validate results.

The limitation of this study is its cross-sectional design, which allowed us to use only one measurement for each variable and didn't allow for the follow-up of patients. As CSVD is a chronic and commonly asymptomatic disease, it might take years for patients to develop LAAIS, making the prospective aspect of the study less feasible. However, our findings might serve as a motivation to engage in such a study, which would be necessary in order to confirm our results and definitely establish predictors for LAAIS in CSVD patients. Another limitation comes from the data availability. As mentioned, the hospital went through a couple of changes in the way data is stored over the period this study included, therefore, some data was lost and unavailable. In order to be as transparent as possible, we presented data availability for each variable in Supplementary Table 2 and discussed the limitations of data collection and availability for each variable where we found it could affect our results. It should be noted that a number of these patients are regularly followed up in the clinic, which will allow for additional data gathering and a follow-up prospective study.

6. CONCLUSIONS

Based on the results from this study, we can conclude the following:

1. **Atherosclerosis marked by ICA atherosclerosis and presence of atherosclerotic CVD, and inflammation marked by elevated NLR are important risk factors for LAAIS in CSVD patients that could be used in evaluating the risk of stroke in this population.**

Among all risk factors, atherosclerosis (ICA and atherosclerotic CVD) and inflammation (NLR) had the highest OR and RR, while the BBB dysfunction (CSF/serum albumin ratio) had a slightly elevated RR. Atherosclerosis evaluation in the form of a non-invasive extracranial or intracranial artery examination, or coronary artery imaging, and NLR measurement might be useful when evaluating CSVD patients to determine the risk of stroke.

2. **The CSVD MRI burden score is a quick and clinically available method to evaluate the disease burden and the risk of LAAIS in CSVD patients.**

Our study showed that patients with an increased CSVD burden are at a higher risk for LAAIS. This scoring system can be easily calculated from the patients' MRI scans and used clinically. However, more work is needed to standardize the method.

3. **The CSVD MRI burden score severity is correlated with the degree of atherosclerosis and inflammation.**

Regarding the CSVD burden score, we confirmed the previous notion that hypertension is involved in CSVD and its progression, while showing the involvement of atherosclerosis and inflammation.

4. **Sex has no influence on CSVD progression or LAAIS occurrence.**

Sex differences shown in this study were related to physiological differences, risk factors, or reflected different lifestyles, but not involved in CSVD or LAAIS.

5. **"Traditional" cerebrovascular risk factors are not useful in determining the risk of stroke in CSVD patients, possibly due to their high prevalence in both conditions.**

Other "traditional" cerebrovascular risk factors didn't show an association with LAAIS in CSVD patients. This probably stems from the fact that they are involved in both conditions and therefore present in high prevalence in our cohort.

6. **Total cholesterol and HDL-C were inversely correlated with LAAIS in CSVD patients, which could be specific to this patient population.**

Both total cholesterol and HDL-C have shown an inverse correlation to LAAIS, indicating that increased values favor small vessel disease and LS development more than LAAIS, shifting the balance in our cohort.

7. **ICA stenosis, CSVD burden on imaging, and inflammation are more severe in LAAIS than in LS.**

We showed that ICA stenosis and the CSVD burden were more severe in LAAIS than LS, confirming the influence of atherosclerosis on LAAIS. LS were less severe and had less inflammation, which, we believe, stems from their pathological mechanism. Total cholesterol values were different between these two groups, confirming that high total cholesterol itself predisposes patients to symptomatic LS more than LAAIS.

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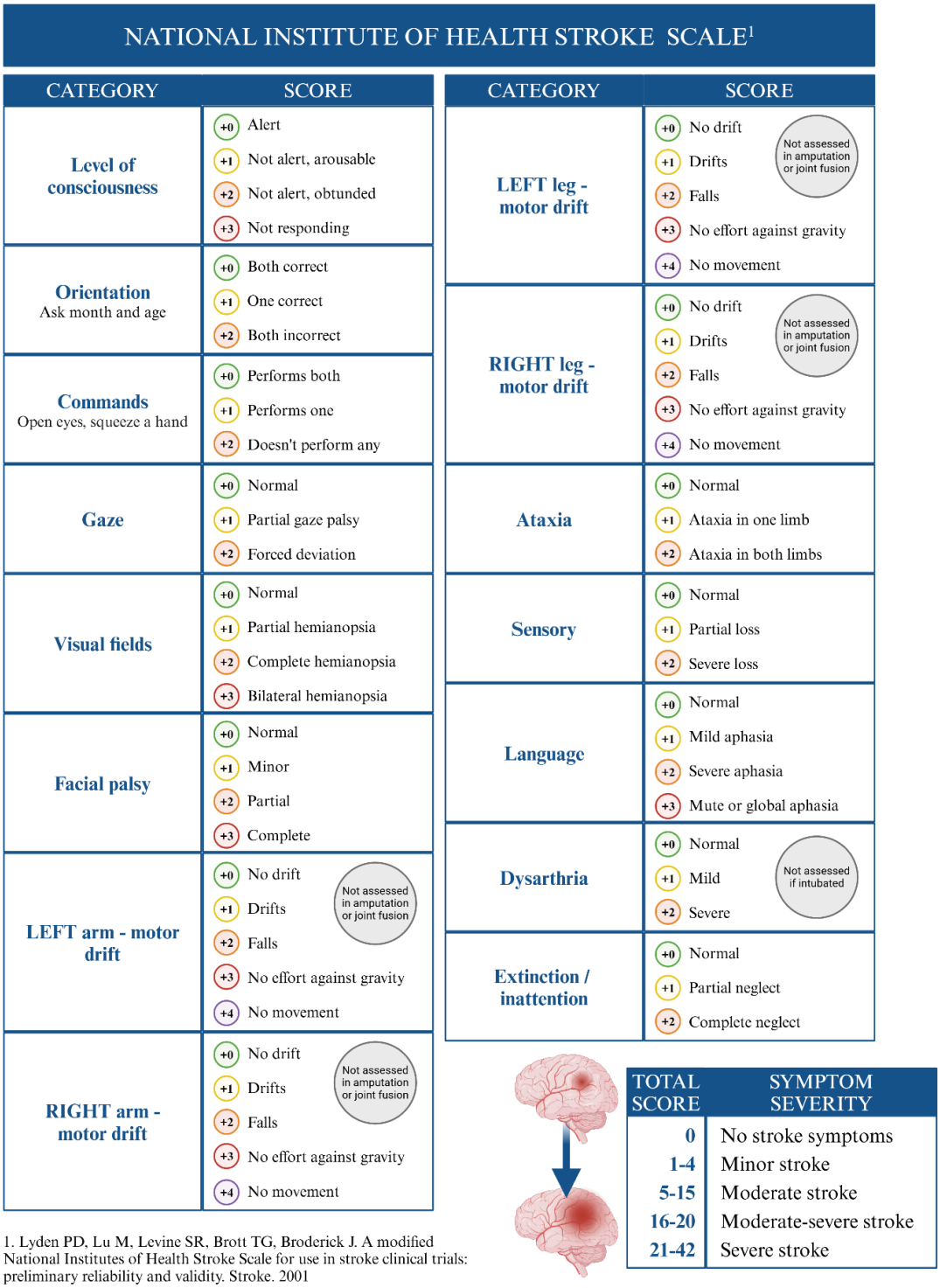
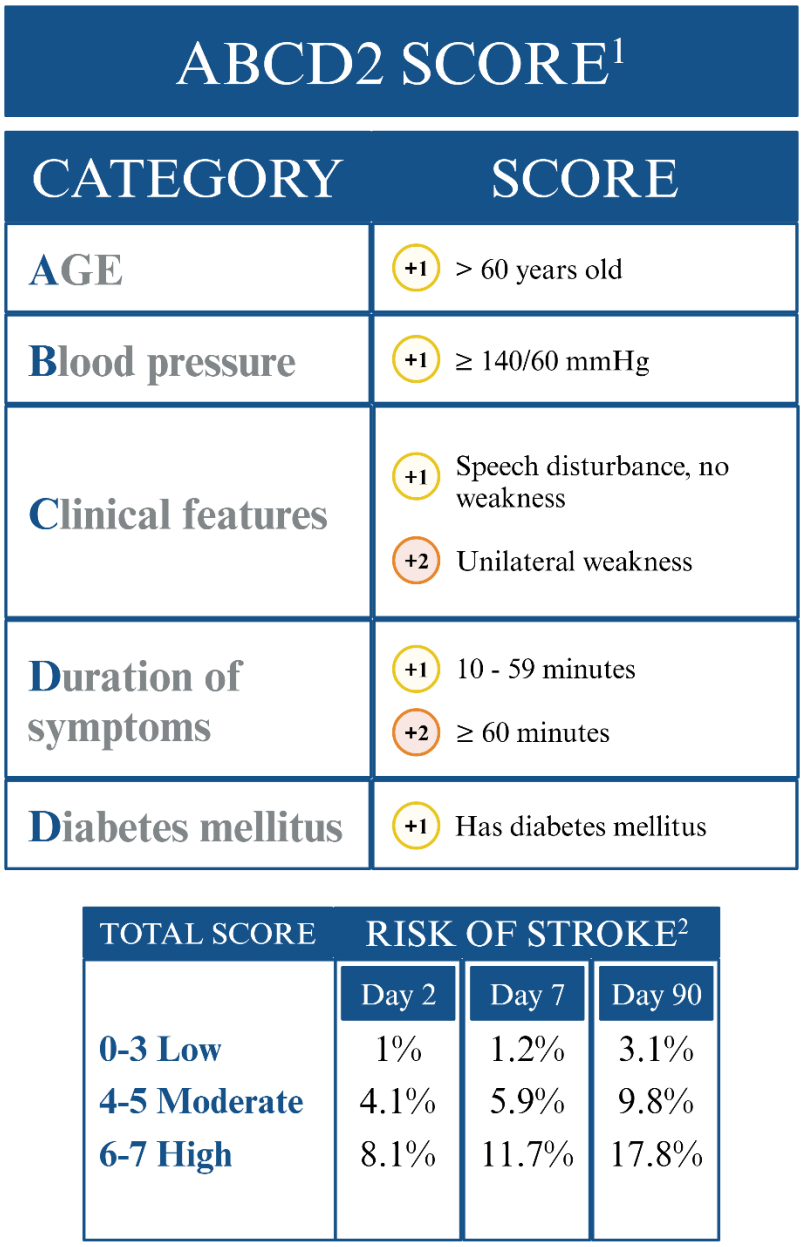
List of used abbreviations

ACA	Anterior cerebral artery
AIS	Acute ischemic stroke
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
aPTT	Partial thromboplastin time
AST	Aspartate aminotransferase
BA	Basilar artery
BBB	Blood-brain barrier
BHI	Breath-holding index
CAA	Cerebral amyloid angiopathy
CAD	Coronary artery disease
CADASIL	Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy
CARASIL	Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy
CBC	Complete blood count
CI	Confidence interval
CKD	Chronic kidney disease
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CSVD	Cerebral small vessel disease
CT	Computed tomography
CVD	Cardiovascular disease
DAPT	Dual antiplatelet therapy
DM	Diabetes mellitus
DWI	Diffusion-weighted imaging
EDV	End-diastolic velocity
eGFR	Estimated glomerular filtration rate
EPVS	Enlarged perivascular spaces
ESO	European Stroke Organization
FLAIR	Fluid-attenuated inversion recovery
fT3	Free triiodothyronine
fT4	Free thyroxine
FV	Flow velocity
GGT	Gamma-glutamyl transferase
GRE	Gradient recalled echo
HbA1C	Glycosylated hemoglobin A1c
HDL-C	High-density lipoprotein cholesterol
Hgb	Hemoglobin
HR	Hazard ratio
ICA	Internal carotid artery
IL	Interleukin
IMT	Intima-media thickness
INR	International normalized ratio
IQR	Interquartile range
LAAIS	Large artery acute ischemic stroke
LDL-C	Low-density lipoprotein cholesterol
LS	Lacunar strokes

MACCE	Major adverse cardiac and cerebrovascular events
MCA	Middle cerebral artery
MCI	Mild cognitive impairment
MFV	Mean flow velocity
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NIHSS	National Institutes of Health Stroke Scale
NLR	Neutrophil to lymphocyte ratio
NMDA	N-methyl-D-aspartate
OR	Odds ratio
PAD	Peripheral arterial disease
PCA	Posterior cerebral artery
PI	Pulsatility index
PMN	Polymorphonuclear leukocytes
PSV	Peak systolic velocity
PT	Prothrombin time
RBC	Red blood cells
RR	Relative risk
SAPT	Single antiplatelet therapy
SGLT1	Sodium-glucose cotransporter 1
SGLT2	Sodium-glucose cotransporter 2
STRIVE	Standards for Reporting Vascular Changes on Neuroimaging
TCD	Transcranial color Doppler
TIA	transient ischemic attack
TOAST	Trial of Org 10172 in acute stroke treatment
tPA	Tissue plasma activator
TSH	Thyroid-stimulating hormone
VA	Vertebral artery
VB	Vertebrobasilar
VMR	Vasomotor reactivity
WBC	White blood cells
WMH	White matter hyperintensities



Supplementary Figure 1. NIHSS scoring used in the study, based on the previously validated method (142) Created in Biorender.com



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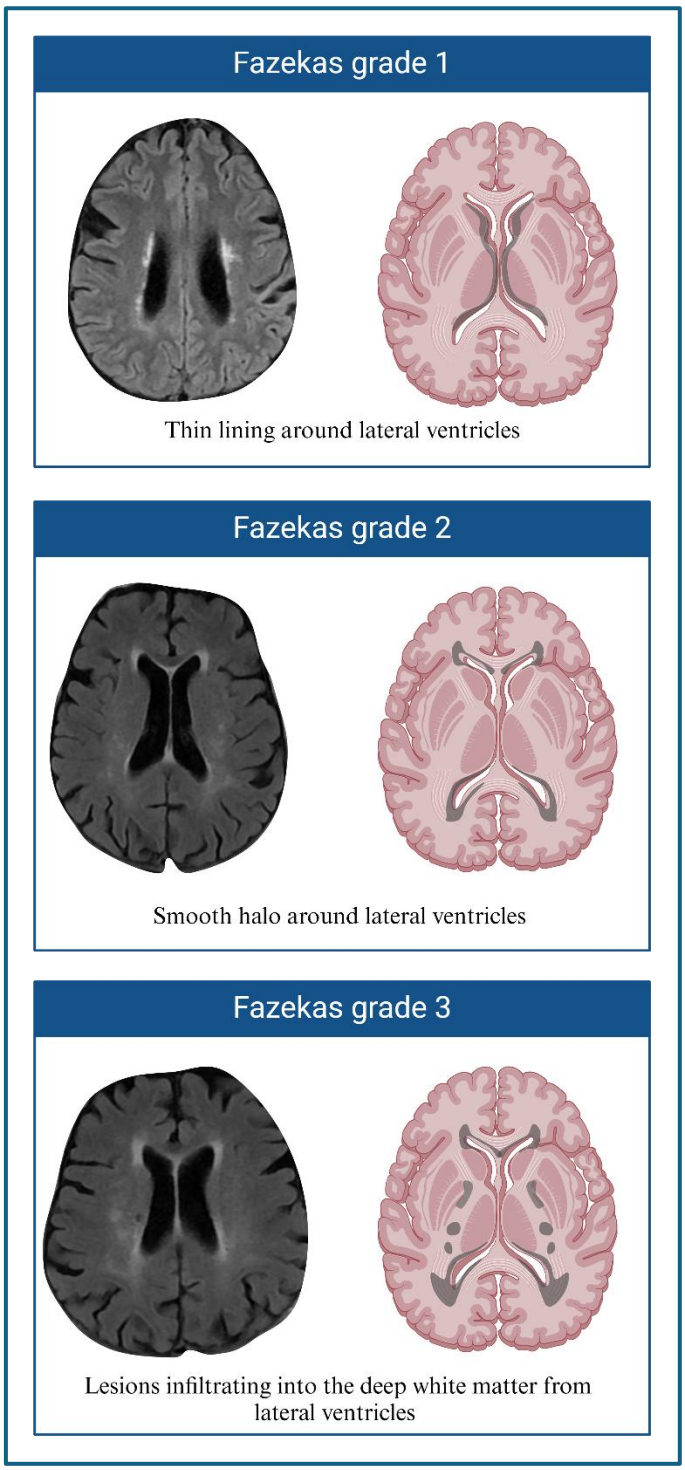
Supplementary Figure 2. ABCD2 scoring used in the study, based on the previously validated method (143,144) Created in Biorender.com



Supplementary Figure 3. Modified Fazekas scale used for grading WMH, based on the previously validated method (97)

Supplementary Table 1. Reference ranges for each laboratory parameter used in the study

Parameter	Reference range	Units
Total cholesterol	< 5.2	mmol/L
LDL-C	< 3.4	mmol/L
Triglycerides	< 1.7	mmol/L
RBC	Women: 3.86-5.05 Men: 4.34-5.72	10^12/L
WBC	3.4-9.7	10^9/L
Neutrophils	2.1-6.5	10^9/L
Lymphocytes	1.2-3.4	10^9/L
PLT	158-424	10^9/L
Hgb	Women: 119-157 Men: 138-175	g/L
Glucose	6.1	mmol/L
HbA1c	6.5	%
Urea	2.5-7.5	mmol/L
Creatinine	45-84	µmol/L
eGFR	>60	mL/min/1.73m2
Serum proteins	62-81	g/L
Albumin	35-53	g/L
B12	187-883	pg/L
Folic acid	3.1-20.5	mmol/L
Homocysteine	5.0-15.0	µmol/L
ALP	40-120	U/L
AST	0-37	U/L
ALT	0-41	U/L
GGT	0-38	U/L
CRP	0-5	mg/L
Fibrinogen	2.0-4.0	g/L
D-dimer	< 0.5	µg/ml
Antithrombin	83-118	%
Plasminogen	75-150	%
Protein C	70-140	%
PT	10.4-13.0	s
aPTT	22-32	s
INR	0.8-1.2	
Factor II	70-120	%
Factor V	70-120	%
Factor VIII	70-120	%
Factor X	70-120	%
Factor XI	70-120	%

Parameter	Reference range	Units
Factor XII	70-150	%
Factor XIII	70-140	%
Lupus anticoagulant 1	31-41	s
Lupus anticoagulant 2	26-31	s
Von Willebrand Factor	48-173	%
TSH	0.35-4.94	µIU/ml
fT3	2.63-5.7	pmol/L
fT4	9.0-19.1	pmol/L
CSF glucose	2.7-4.1	mmol/L
CSF albumin	0.15-0.45	g/L

Abbreviations: LDL-C – low-density lipoprotein cholesterol, RBC – red blood cells, WBC – white blood cells, PLT – platelets, Hgb – hemoglobin, HbA1c – glycosylated hemoglobin A1c, eGFR – estimated glomerular filtration rate, ALP – alkaline phosphatase, AST – aspartate aminotransferase, ALT – alanine aminotransferase, GGT – gamma-glutamyl transferase, CRP – C-reactive protein, PT – prothrombin time, aPTT – partial thromboplastin time, INR – international normalized ratio, TSH – thyroid stimulating hormone, fT3 – free triiodothyronine, fT4 – free thyroxine, CSF – cerebrospinal fluid.

Supplementary Table 2. Data availability for each variable used in the study

Characteristic	N	Characteristic	N
Gender	241	Left VA EDV	241
Age	241	TCD anterior stenosis	68
ICA plaque	241	TCD VB stenosis	93
Right ICA Plaque	241	TCD anterior low flow	68
Right ICA Stenosis	241	TCD VB low flow	93
Right ICA stenosis degree	241	Right ACA MFV	68
Left ICA Plaque	241	Right ACA PI	68
Left ICA Stenosis	241	Right MCA MFV	68
Left ICA stenosis degree	241	Right MCA PI	68
Sum stenosis	241	Right PCA MFV	68
Right IMT	241	Right PCA PI	68
Left IMT	241	Left ACA MFV	68
Sum IMT	241	Left ACA PI	68
Right ICA variation	241	Left MCA MFV	68
Left ICA kink variation	241	Left MCA PI	68
Right ICA PSV	241	Left PCA MFV	68
Right ICA EDV	241	Left PCA PI	68
Left ICA PSV	241	Right VA MFV	93
Left ICA EDV	241	Right VA PI	93
Right VA Plaque	241	Left VA MFV	93
Left VA Plaque	241	Left VA PI	93
Right VA hypoplasia	241	BA1 MFV	93
Left VA hypoplasia	241	BA1 PI	93
Right VA PSV	241	BA2 MFV	93
Right VA EDV	241	BA2 PI	93
Left VA PSV	241	Right BHI	25
Left BHI	24	WBC	241
Imaging	241	Neutrophils	178
LS	241	Lymphocytes	178
LS localization	217	NLR	178

Characteristic	N	Characteristic	N
WMH	241	PLT	241
WMH Fazekas scale	75	Hgb	241
Microbleeds	196	Glucose	241
EPVS	196	HbA1c	52
EPVS category	64	Urea	234
Atrophy	241	Creatinine	233
CSVD MRI burden score	196	eGFR	140
Large artery AIS	241	Kidney dysfunction	241
Side	52	Serum Proteins	231
Artery	52	Albumin	225
TOAST	116	B12	160
NIHSS	97	Folic Acid	122
TIA	241	Homocysteine	127
ABCD2	35	ALP	197
Atherosclerotic CVD	241	AST	234
Total cholesterol	241	ALT	233
LDL-C	203	GGT	221
HDL-C	210	CRP	213
Triglycerides	236	Fibrinogen	225
Dyslipidemia	241	D-dimer	202
HTN	241	Antithrombin	61
DM	241	Plasminogen	33
Smoking	192	Protein C	67
RBC	241	PT	241
aPTT	241	TSH	183
INR	241	fT3	124
Factor II	78	fT4	180
Factor V	77	CSF Glucose	83
Factor VII	75	CSF Albumin	82
Factor VIII	69	CSF PMN	83
Factor IX	73	CSF WBC	83

Characteristic	N	Characteristic	N
Factor X	66	CSF RBC	83
Factor XI	73	CSF/serum albumin ratio	82
Factor XII	74		
Factor XIII	43		
Lupus anticoagulant	61		
VonWillebrand Factor	47		

Abbreviations: ICA – internal carotid artery, IMT – intima-media thickness, PSV – peak systolic velocity, EDV – end diastolic velocity, VA – vertebral artery, TCD – transcranial color Doppler, ACA – anterior cerebral artery, MCA – middle cerebral artery, PCA – posterior cerebral artery, BA – basilar artery, MFV – mean flow velocity, PI – pulsatility index, BHI – breath-holding index, LS – lacunar stroke, WMH – white matter hyperintensity, EPVS – enlarged perivascular spaces, CSVD – cerebral small vessel disease, MRI – magnetic resonance imaging, AIS – acute ischemic stroke, TOAST – Trial of Org 10172 in acute stroke treatment, NIHSS – National Institutes of Health Stroke Scale, TIA – transient ischemic attack, ABCD2 – Age, Blood pressure, Clinical features, Duration, Diabetes, CVD – cardiovascular disease, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol, HTN – hypertension, DM – diabetes mellitus, RBC – red blood cells, WBC – white blood cells, NLR – neutrophil to lymphocyte ratio, PLT – platelets, Hgb – hemoglobin, HbA1c – glycosylated hemoglobin A1c, eGFR – estimated glomerular filtration rate, ALP – alkaline phosphatase, AST – aspartate aminotransferase, ALT – alanine aminotransferase, GGT – gamma-glutamyl transferase, CRP – C-reactive protein, PT – prothrombin time, aPTT – partial thromboplastin time, INR – international normalized ratio, TSH – thyroid stimulating hormone, fT3 – free triiodothyronine, fT4 – free thyroxine, CSF – cerebrospinal fluid, PNM – polymorphonuclear cells.

Biography

Stefan Stoisavljević graduated from the University of Belgrade, Faculty of Medicine in 2020, gaining the degree of Doctor of Medicine (MD). In the same year, Stefan enrolled in the University of Belgrade, Faculty of Medicine as a PhD student in Medicine (Neurology), under the supervision of Professor Milija Mijajlović, MD, PhD. The first research papers, Stefan wrote during his medical studies, one of which was focusing on cerebral small vessel disease, which carried over into his PhD degree.

During his doctoral studies, from September 2023. to August 2024. Stefan attended the Karolinska Institute in Stockholm, Sweden, sponsored by the Erasmus+ mobility program, under the supervision of Associate Professor Joy Roy. During this time, Stefan attended additional courses relevant to his education and enriched his research experience.

Stefan Stoisavljević performed his medical doctor internship at the Clinical Center of Serbia from October 2020. to March 2021. After that, he passed the medical license examination and became a member of the Serbian Medical Chamber. In 2024, Stefan became a member of the Society of Serbian Neurologists.

As of June 2017. Stefan started working part-time with Osmosis from Elsevier as a medical writer, focusing on creating educational content for medical students. In 2022, he became a full-time senior medical content editor.

Publications resulting from the thesis

1. Stoisavljevic S, Zdraljevic M, Radojicic A, Pavlovic A, Mijajlovic M. Carotid artery stenosis is related to cerebral small vessel disease magnetic resonance imaging burden. Heliyon. 2024;10(16):e36052. doi:10.1016/j.heliyon.2024.e36052

2. Stoisavljevic S, Stojanovic M, Zdraljevic M, Aleksic V, Pekmezovic T, Mijajlovic M. Correlation between Morphological and Hemodynamic Parameters of Carotid Arteries and Cerebral Vasomotor Reactivity. Brain Sci. 2024;14(2):167. doi:10.3390/brainsci14020167

3. Stoisavljevic S, Mijajlovic M. The Use of Transcranial Doppler Microembolic Signals Detection in Carotid Artery Stenosis. Medicinski podmladak. 2027;78(1). doi: 10.5937/mp78-52229

образац изјаве о ауторству

Изјава о ауторству

Име и презиме аутора Стефан Стоисављевић

Број индекса 2020/5080

Изјављујем

да је докторска дисертација под насловом

Разлике у учесталости васкуларних фактора ризика код болесника са болешћу малих крвних судова мозга са и без последичног исхемијског можданог удара

Differences in Vascular Risk Factor Frequency Among Patients with Cerebral Small Vessel Disease with and without Consequential Ischemic Stroke

- резултат сопственог истраживачког рада;
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Потпис аутора

У Београду, 20.01.2025.

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образац изјаве о истоветности штампане и електронске верзије докторског рада

Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора Стефан Стоисављевић

Број индекса 2020/5080

Студијски програм Неурологија

Наслов рада

Разлике у учесталости васкуларних фактора ризика код болесника са болешћу малих крвних судова мозга са и без последичног исхемијског можданог удара

Differences in Vascular Risk Factor Frequency Among Patients with Cerebral Small Vessel Disease with and without Consequential Ischemic Stroke

Ментор Доц. др Милија Мијајловић

Изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла ради похрањивања у **Дигиталном репозиторијуму Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског назива доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

Потпис аутора

У Београду, 20.01.2025.

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образац изјаве о коришћењу

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

Разлике у учесталости васкуларних фактора ризика код болесника са болешћу малих крвних судова мозга са и без последичног исхемијског мождано удара

Differences in Vascular Risk Factor Frequency Among Patients with Cerebral Small Vessel Disease with and without Consequential Ischemic Stroke

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигиталном репозиторијуму Универзитета у Београду и доступну у отвореном приступу могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

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