

Blood-Based Biomarkers Could Help Identify Subclinical Brain Damage Caused by Arterial Hypertension

Alina González-Quevedo MD PhD, Sergio González-García PhD, Marisol Peña-Sánchez MS, Caridad Menéndez-Sainz PhD, Rebeca Fernández-Carrera MS, Anay Cordero-Einz MS

ABSTRACT

Arterial hypertension is the most prevalent non-communicable disease worldwide, and has long been recognized as a major risk factor for cardiovascular and cerebrovascular diseases. High blood pressure has deleterious consequences on the main target organs (heart, kidney, brain), and several studies have shown that brain damage is more frequent than heart and kidney involvement. Silent lesions can subsequently lead to cognitive decline, dementia or stroke.

Nevertheless, screening for subclinical brain deterioration is rarely performed because it requires imaging techniques whose scarcity and high cost rule out routine use by primary care physicians. The challenge is thus early detection of asymptomatic brain lesions with cost-effective techniques to test thousands of patients in the community. In this review we present an update on the status of biomarkers explored as alternatives for early detection of brain damage in arterial

hypertension, potentially useful to identify patients needing referrals for brain MRI: ambulatory blood pressure monitoring, quantitative retinal microvascular assessment, quantitative electroencephalography, carotid ultrasonography, neurocognitive studies and blood-based biomarkers. We place special emphasis on blood-based biomarkers, for which our group reported the first preliminary evidence of an association between serum neuron-specific enolase and severity of white matter lesions in patients with essential hypertension. This review consequently explores the potential for blood-based biomarkers to provide a faster, cheaper and more accessible early-detection solution, particularly beneficial in resource-limited settings such as Cuba's.

KEYWORDS Arterial hypertension, small vessel disease, brain damage, biomarkers, white matter diseases, leukoaraiosis, lacunar infarct, S100 calcium binding protein beta subunit, S100B protein, S100 calcium binding protein G, neuron specific enolase, NMDA receptors, receptors, N-methyl-D-aspartate, mass screening, Cuba

INTRODUCTION

Arterial hypertension (HT) is the most prevalent non-communicable disease worldwide, long recognized as a major risk factor for cardiovascular and cerebrovascular disease.[1] It has been associated with a 62% risk of cerebrovascular disease, such as ischemic stroke, and 49% risk of cardiovascular disease.[2] A recent meta-analysis revealed that stroke risk increased with the severity of prehypertensive status.[3]

HT management includes assessment of target organ damage (i.e., heart, blood vessels, kidneys and brain), because multi-organ damage is associated with worse prognosis. But in practice, less than 15% of clinicians investigate brain damage caused by HT.[4]

The brain is an early target for organ damage due to HT.[1,5] Subclinical or "silent" vascular brain lesions are often detected by neuroimaging in asymptomatic patients. These can subsequently lead to cognitive decline, dementia or stroke.[6] Thus, early detection of such lesions could identify patients at higher risk for stroke, dementia and cardiovascular events, who could benefit from more aggressive preventive strategies.

Since HT is so common, clinicians look carefully for signs of organ involvement; a large body of evidence is now available on the crucial role of subclinical organ damage.[1,4,7] well established, for cardiovascular and renal diseases because the techniques applied are widely available. In contrast, detection of early brain deterioration requires imaging techniques too scarce and costly for routine use in general practice.

The challenge is to detect asymptomatic brain lesions early with cost-effective techniques for mass population screening. This article reviews the current status of biomarkers for early detection of brain damage in HT, with emphasis on use of blood-based biomarkers, presenting as well our experience and thinking on serum biochemical markers of brain injury.

Our objective was to review the various biomarkers used for early detection of brain damage in HT, and to assess their usefulness and feasibility of routine use. In particular, blood-based biomarkers were explored due to their potential for providing a more accessible, rapid and less expensive solution, requiring minimal expertise to interpret.

EVIDENCE ACQUISITION

Studies were identified through PubMed, SciELO and Cochrane databases through March 30, 2015 using the following search terms: hypertension AND brain damage AND biomarkers, without language restrictions. Published studies that evaluated subclinical brain damage in patients with HT employing different biomarkers (brain imaging, ambulatory blood pressure monitoring, quantitative retinal microvascular assessment, quantitative electroencephalography, carotid ultrasonography, neurocognitive studies and blood-based biomarkers) were included.

Because subclinical white matter lesions (WML) have other risk factors besides HT (age, diabetes mellitus and others),[6] we also included studies of age and diabetes mellitus, and community-based studies of associations between asymptomatic WML and blood-based biomarkers. References in retrieved articles were also reviewed to search for additional pertinent sources.

RESULTS

Hypertension and subclinical brain damage HT can surreptitiously damage the body for years before symptoms develop, through an event cascade that thickens and stiffens artery walls throughout the body, blocking blood flow to the heart, kidneys, brain and limbs. This silent and progressive damage over decades subsequently leads to peripheral artery disease and/or damage to heart, kidneys and brain.[8,9]

Continuous blood pressure changes in the brain cause vascular remodeling, hemodynamic alterations, increased vascular resis-

tance and endothelial dysfunction, all related to tissue damage, revealed as silent lacunar infarcts, microbleeds and white matter changes. Chronic ischemia or acute disruption of blood supply to brain tissue can further trigger mild cognitive impairment, dementia, transient ischemic attacks or stroke.[10,11]

A population study of 3600 participants (free of stroke or transient ischemic attack, mean age 65 years) using MRI found only 4.4% free of any white matter abnormalities. That is, the vast majority of the population had degenerative changes, which were associated with impaired cognitive and lower extremity function.[12]

Silent cerebral WML are frequently found on brain MRI in older adults. However, they tend to occur earlier and appear to be more severe in patients with HT.[13] In the Atherosclerosis Risk In Communities Study (ARIC) of 1920 persons aged 55–72 years, without history of stroke or transient ischemic attack, the prevalence of MRI-defined WML was 85%, associated with HT duration, treatment and control.[14] In the Cardiovascular Health Study, WML prevalence was 87% in HT patients.[15] Lower WML prevalence has been reported by van Boxtel in 65% of HT patients with a mean age of 57.4 years.[16] and Henskens in 44% of a younger cohort (mean age 51.6 years).[7] In one Cuban study of HT patients with a mean age of 44.2 years, classical silent brain lesions were detected in 40%;[17] while our group reported silent WML in 73.9% of an older cohort (mean age 59 years) of essential HT patients,[18] and more recently, classical silent MRI-detected brain lesions in 70.6% of essential HT patients.[19] In sum, WML frequency increases with age.

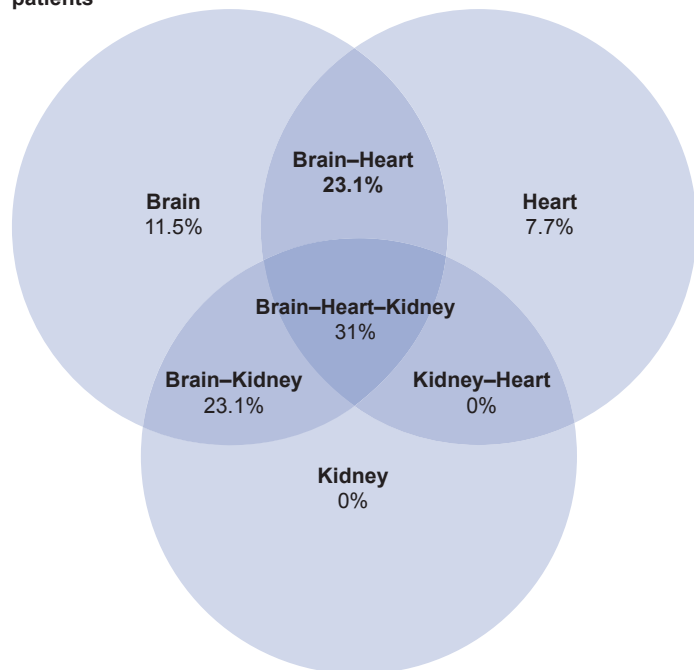
Henskens showed that silent cerebrovascular damage was more common than cardiorenal impairment in essential HT patients.[7] The 2013 European HT guidelines[1] recommend searching for asymptomatic organ damage, more specifically cardiovascular and chronic kidney disease; even though they acknowledge that brain MRI studies show that silent cerebrovascular lesions are even more prevalent (44%) than cardiac (21%) and renal (26%) subclinical damage and frequently occur in the absence of other signs of organ damage. We assessed the three main target organs (heart, kidney and brain) in a group of asymptomatic essential HT patients and found the brain by far the most frequently affected (77.8%), followed by heart (59.2%) and kidney (55.5%). Silent brain damage without cardiorenal impairment was observed in 11.5% (Figure 1).[19]

Van Dijk demonstrated that systolic and diastolic blood pressure were positively associated with WML progression, in patients aged 60–90 years, new lesions occurring in up to 40% of patients.[20] This effect on brain lesion progression was stronger in younger patients and remained after adjusting for use of antihypertensive medications.[20] Gottesman found a similar pattern for midlife patients.[21]

While the entire vasculature is affected by HT, its particular effects on small vessels are key in the severity of damage to cerebral structures.[8,13] Perforating vessels—which originate in the cortical and leptomeningeal arteries—have a relatively poor anastomotic system, making white matter particularly vulnerable to blood pressure changes, so both low and high blood pressure have been reported as WML risk factors.[8]

Sierra hypothesized that long-standing HT causes lipohyalinosis of the media and thickening of vessel walls, with luminal narrowing

Figure 1: Target organ damage detected in 34 asymptomatic HT patients



Source: González-García,[19] used under terms of Creative Commons Attribution 4.0 license
HT: hypertension

of the small perforating arteries and arterioles that nourish the deep white matter, developing the changes that lead to WML.[22]

WML are also related to mild cognitive impairment in cognitively intact older adults.[23] In HT patients aged 40–88 years, prevalence of silent brain infarcts ranges from 20%–86% and is related to stroke and cognitive decline.[20] The multiethnic Northern Manhattan Study (NOMAS) examined a clinically stroke-free population and found 17.7% had subclinical brain infarcts, prevalence increasing with age.[24] Both subclinical infarcts and white matter hyperintensity volume were associated with a globally worse cognitive performance.[25] Delgado recently reported a 10.1% prevalence of silent brain infarcts in a cohort of 976 essential hypertensive patients aged 50–70 years.[26] The high prevalence of silent brain infarcts in the population and their clinical consequences led the American Heart Association to include them in their 2013 updated stroke definition.[27]

Research on the third generation of the Framingham Heart Study found accelerated brain aging in HT and prehypertensive individuals in their 40s, including damage to the structural integrity of the brain's white and gray matter. They concluded that subtle vascular brain injury caused by raised blood pressure develops gradually, with noticeable effects even in young adults. Their findings also revealed significant brain-cell damage in people with HT decades prior to the appearance of dementia symptoms.[2] emphasizing the need for early and optimal blood pressure control.

The foregoing supports the contention that brain damage in the form of WML and silent brain infarcts can be present in asymptomatic HT patients,[1,7] highlighting the importance of assessing subclinical brain injury in HT patients, especially those with cognitive changes.

Assessment of subclinical brain injury in HT patients The gold standard for assessing early brain damage in HT is brain MRI, although positron emission tomography (PET) has also been employed.[7,28] Recent advances in MRI methods using contrast agents provide additional opportunities to measure events in the cerebral vasculature, such as blood–brain barrier rupture, leukocyte infiltration, cell adhesion, molecule upregulation and vascular remodeling.[29]

While assessment of HT's cardiorenal effects has been routine clinical practice for years, not so for HT neurological damage due to limited availability of MRI and cost considerations. Thus, alternative methods for demonstrating asymptomatic neurological damage by HT are needed.

Other more accessible and less expensive techniques to assess risk of subclinical brain damage have been proposed, such as ambulatory blood pressure monitoring,[16,24] quantitative retinal microvascular assessment,[30,31] quantitative electroencephalography,[17,32] carotid ultrasonography[19] and neurocognitive studies.[9]

Ambulatory blood pressure monitoring Twenty-four hour ambulatory blood pressure monitoring (ABPM) has become an important tool for improving HT diagnosis and management. Several studies have demonstrated an association between 24-h ABPM results and white matter hyperintensities in HT patients.[16,22,33,34] However most of these studies have been cross-sectional, so any conclusions about the direction of the relationship between ABPM and WML remain speculative. Longitudinal studies are required to establish causality.

Steady and pulsatile components of daytime, nighttime and 24-h blood pressure have gained increased interest in the prediction of WML, lacunar infarctions and stroke. Van Boxtel reported no association between nighttime dipping of blood pressure and white matter hyperintensity load, whereas daytime and 24-h pulse pressure averages were associated with paraventricular WML, and systolic blood pressure and mean arterial pressure for all periods were higher in patients with lacunar infarctions. He concluded that there was no relationship between diurnal blood pressure rhythm and signs of structural or functional cerebral damage in a population of newly diagnosed HT individuals, but that ABPM profiles may predict lesion type in early asymptomatic cerebral abnormalities.[16]

It remains unknown whether and to what extent ABPM could prove useful for detecting subclinical central nervous system damage in HT patients. Notwithstanding, in 2014 Avet's PROOF study of 830 individuals aged 65±1 years found relationships between total WML score and 24-hour systolic blood pressure, 24-hour diastolic blood pressure, daytime systolic and diastolic blood pressure, and nighttime systolic blood pressure; WML score was not associated with nocturnal blood pressure dip, nor with other recognized cardiovascular risk factors. They identified a mean 24-hour systolic blood pressure threshold of 123 mmHg for identifying patients at high-risk for WML.[34]

Quantitative retinal microvascular assessment The retina offers a unique, noninvasive and easily accessible window to study the microvascular etiology of cerebrovascular disease. HT retinal changes defined qualitatively from a fundus examination have been traditionally classified into four grades of retinopathy;[35] nevertheless, most HT patients today are diagnosed early in the

process of their illness, and hemorrhages and exudates (grade 3) and papilledema (grade 4), are observed very rarely. Milder degrees of retinopathy appear to be largely nonspecific arteriolar alterations (grades 1 and 2) and their usefulness for prognosis has been questioned.[5]

Thus, more selective methods for objectively investigating ocular HT damage have been developed and studied. Digitized retinal photography has been used to quantify the geometric and topological properties of arteriolar and venous trees, demonstrating that retinal arteriolar and venous narrowing may precede HT development.[31]

Cheung developed new quantitative and qualitative parameters to describe retinal branching patterns and improve sensitivity and specificity in detecting retinal microcirculatory alterations. This approach provided interesting data on quantitative and qualitative assessment of retinal microvascular abnormalities in a general population and their relationships with blood pressure values.[30] Scuteri related presence of WMLs not only to elevated brachial systolic blood pressure, pulse pressure and arterial stiffness, but also to retinal arterial narrowing.[9]

Retinal microvascular flow is known to be reduced in persons with WML and lacunar infarction. In a cohort of 1684 asymptomatic individuals aged 51–72 years from the ARIC study, those with WML were more likely to have retinal microvascular abnormalities and at greater long-term risk of subclinical cerebrovascular disease on MRI, independent of conventional risk factors. The ARIC authors suggested that retinal microvascular abnormalities are early and possibly more sensitive markers of subclinical cerebral small vessel disease than imaging techniques.[36]

Although quantitative retinal microvascular assessment seems promising as a biomarker for predicting early brain damage in HT patients, one obstacle to its translation to routine clinical practice is the need for accurate documentation and measurement of retinal arteriolar narrowing, which requires highly specialized and automated equipment not yet commonly available in primary care settings.

Quantitative electroencephalography Another line of investigation has been brain electrical activity in HT. A study in neurologically asymptomatic subjects with essential HT found that quantitative EEG revealed altered spontaneous brain activity, mainly in the frontal and midline regions of the left hemisphere, which the authors interpreted as due to brain hypoperfusion.[32] The same researchers later used low-resolution electromagnetic tomography to investigate abnormal electrical activity in 84 hypertensive patients with no history of neurological disease.[37] They found that the electrical sources were located mainly in right Brodmann areas 17 and 37, and left Brodmann areas 6, 10 and 39, regions that are very sensitive to hypoperfusion secondary to HT. Although these studies revealed impairment of brain electrical activity in neurologically asymptomatic HT patients, neither associated quantitative EEG findings with brain MRI lesions.[32,37]

Hernández assessed the diagnostic accuracy of a broad battery of tests for predicting subclinical brain MRI lesions in a pilot study of 50 HT patients with no known neurological disease (excluded from the Cuban Human Brain Mapping Project solely because of HT), and noted that quantitative EEG predicted more severe MRI lesions, with almost 91% diagnostic accuracy.[17] Brown assessed the results of conventional EEG in the same patients,

quantifying observations from visual inspection employing the grand total EEG scale, and found an association between severity of EEG changes and brain MRI lesions.[38]

Carotid ultrasonography There are very few studies correlating structural and hemodynamic carotid ultrasound parameters with extent of brain MRI lesions in asymptomatic HT patients, and the results are controversial. The most frequently examined variable in extracranial ultrasound has been intima-media thickness, which was found increased in association with brain MRI lesions.[17,39] In a cohort of 1684 asymptomatic persons aged 51–72 years from the ARIC study, individuals with WML had increased carotid intima-media thickness.[36] However, Heliopoulos found no such association in a sample of 52 HT patients (mean age 71.4 years).[40] Arterial stiffness has also been associated with microvascular brain damage.[9] Maillard found systolic blood pressure linearly associated with markers of cerebral white matter microstructural damage in young adults.[2] In turn, high systolic pressure has been considered to be a result of accelerated arterial aging or increased stiffness.[41] Our group recently reported increased resistive index and decreased diastolic velocity in essential HT patients with classical brain MRI lesions, possibly indicating a resistive carotid flow pattern in these patients.[19]

Neuropsychological assessment Neuropsychological methods have demonstrated impairment in HT patients in different cognitive domains (attention, memory, executive function), predominantly affecting executive function, with slower information processing, impaired ability to shift from one task to another, and deficits in working memory (ability to hold and manipulate information).[9] It has been suggested that increased blood pressure may explain HT individuals' deterioration in cognitive functions through small vessel disease, white-matter lesions and endothelial dysfunction.[20] Van Boxtel found an association between presence of WML and poor performance on neuropsychological tests in asymptomatic, never-treated essential HT patients.[16]

There is accumulating evidence that the negative effect of elevated blood pressure on cognitive aging is probably mainly mediated by WMLs. Increased WML volume has been associated with decreased episodic memory and executive function, the association stronger for evolution of existing WMLs than for occurrence of new ones.[42]

Cognitive tests may help detect initial brain deterioration in HT patients, but widespread application is challenging because these are time consuming and require a high degree of skill to interpret.

Blood-based biomarkers Blood withdrawal is a very simple and common procedure for assessing numerous cellular and biochemical parameters in medical conditions. Some of these have been explored in connection with subclinical brain damage in asymptomatic individuals. WML and silent cerebral infarcts are the MRI imaging expression of cerebral small vessel disease, whose main risk factor is arterial HT, but also includes others such as age and diabetes mellitus.[6] Mechanisms leading to development of subclinical WML may be different, but the ultimate lesions probably share common molecular indicators of injury in the bloodstream. Thus we consider that information about blood biomarkers of asymptomatic brain damage in individuals with other predisposing conditions may also be of value to appraise changes observed in arterial HT.

Low-grade systemic inflammation participates in the pathophysiology of HT, and C-reactive protein (CRP)—possibly the most

studied blood marker for HT-related inflammation—has been associated with greater risk and severity of stroke in healthy populations[43] and among hypertensive men.[44] Nevertheless, evidence relating inflammatory markers and brain damage is controversial. Table 1 summarizes results reported for association of several blood biomarkers with presence of brain MRI lesions in neurologically asymptomatic individuals. Elevations of the inflammatory markers lipoprotein-associated phospholipase A2 and myeloperoxidase, but not CRP, were found to be associated with a greater burden of WML.[45] Satizabal studied an elderly community-based sample and reported an association between circulating interleukin-6 (IL-6) and CRP levels and more severe WML, but not with lacunar infarcts.[46] while Mitaki revealed opposing results with CRP: an association with silent lacunar infarcts, but not with WML or with cerebral microbleeds.[47] On the other hand, high levels of IL-6 and CRP were reported as useful biomarkers for identifying high risk for periventricular hyperintensities in healthy Japanese subjects.[48]

Shoamanesh's study of a middle-aged, community-based sample (n = 1763) is perhaps one of the most extensive concerning the possible role of inflammation in pathogenesis of cerebral small vessel disease.[49] It assessed the association of a comprehensive panel of 15 systemic inflammatory markers with MRI measures of cerebral small vessel disease. Increased levels of tumor necrosis factor (TNF) receptor 2 and myeloperoxidase were associated with cerebral microbleeds, but not with WML or lacunar infarcts, while higher levels of osteoprotegerin, intercellular adhesion molecule 1 and lipoprotein-associated phospholipase A2, as well as lower myeloperoxidase, were observed in participants with greater WML volumes and silent cerebral infarcts. No associations were encountered between cerebral microbleeds and osteoprotegerin, intercellular adhesion molecule 1 and lipoprotein-associated phospholipase A2; no changes in CRP or TNF alpha levels accompanied brain MRI lesions.

Kim used leukocyte count as a marker of systemic inflammation in his study of 1586 asymptomatic individuals who underwent brain MRI for a routine checkup. He found that elevated blood leukocyte count was associated with moderate to severe WML, independently of known cardiovascular risk factors such as age, HT and diabetes.[50] Additionally, these authors found higher CRP levels related to moderate to severe WML, but this association disappeared after adjustment for leukocyte count, age, and other clinical and demographic variables.

In NOMAS, lower serum levels of soluble receptors for advanced glycation end-products (sRAGE) were associated with higher prevalence of silent brain infarcts and WML in stroke-free Hispanic and black subjects in the USA, respectively, suggesting that sRAGE may be predictive of subclinical cerebrovascular disease, particularly in ethnic/racial groups with increased risk for cerebrovascular disease.[51]

Some biomarkers for cardiovascular disorders have also been explored. Higher N-terminal brain natriuretic peptide and cardiac troponin T were associated with silent brain infarcts and WML, suggesting their usefulness for identifying individuals with subclinical cerebral injury.[52] In addition, a small cross-sectional study by Reinhard revealed that N-terminal brain natriuretic peptide was positively correlated with WML in diabetic patients.[53] Plasma markers of endothelial dysfunction such as intercellular adhesion molecule-1 and P-selectin have also been associated with WML.[54,55]

Table 1: Association of several blood-based biomarkers with brain MRI lesions in neurologically asymptomatic subjects

Biomarker	Author, year	n	Population characteristics	Sample	Country	MRI findings		
						WML	Lacunar infarcts	Micro-bleeds
C-reactive protein	Wright, 2009[45]	527	Stroke free, community based, >55 years	Serum	USA	No change	n/t	n/t
	Satizabal, 2012[46]	1841	Community based, 65–80 years	Plasma	France	Increased	No change	n/t
	Mitaki, 2015[47]	519	Neurologically normal, 29–90 years, mean 63.5 (SD 10.3)	Serum	Japan	No change	Increased	No change
	Abe, 2014[48]	228	Healthy volunteers, 31–83 years, mean 65 (SD 7)	Plasma	Japan	Increased ^a	n/t	n/t
	Shoamanesh, 2015[49]	1763	Community based, mean age 62 years (SD 9.1)	Plasma/serum	USA	No change	No change	No change
	Kim, 2011[50]	1586	Asymptomatic, 20–86 years, mean 53.6	Whole blood	S. Korea	Increased ^b	n/t	n/t
Interleukin-6 (IL-6)	Satizabal, 2012[46]	1841	Community based, 65–80 years	Plasma	France	Increased	No change	n/t
	Abe, 2014[48]	228	Healthy volunteers, 31–83 years, mean 65 (SD 7)	Plasma	Japan	Increased ^a	n/t	n/t
Lipoprotein-associated phospholipase A2 (Lp-PLA2)	Wright, 2009[45]	527	Stroke free, community based, >55 years	Serum	USA	Increased	n/t	n/t
	Shoamanesh, 2015[49]	1763	Community based, mean age 60.2 years (SD 9.1)	Plasma/serum	USA	Increased	Increased	No change
Myeloperoxidase	Wright, 2009[45]	527	Stroke free, community based, >55 years	Serum	USA	Increased	No change	n/t
	Shoamanesh, 2015[49]	1763	Community based, mean age 60.2 years (SD 9.1)	Plasma/serum	USA	Decreased	Decreased	Increased
sRAGE	Hudson, 2011[51]	1102	Stroke free, community based, >55 years	Serum	USA	Decreased ^c	Decreased ^c	n/t
N-terminal brain natriuretic peptide	Dadu, 2013[52]	1501	CVD and stroke free, mean age 65.2 years (SD 4.6)	Plasma	USA	Increased	Increased	n/t
	Reinhard, 2012[53]	20	CVD and stroke free, diabetic, mean age 57 years (SD 10)	Plasma	USA	Increased	n/t	n/t
Cardiac Troponin T	Dadu, 2013[52]	1502	CVD and stroke free, mean age 65.2 years (SD 4.6)	Plasma	USA	Increased	Increased	
TNF receptor 2						No change	No change	Increased
Osteoprotegerin						Increased	Increased	No change
Intercellular Adhesion Molecule 1	Shoamanesh, 2015[49]	1763	Community based, mean age 60.2 years (SD 9.1)	Plasma/serum	USA	Increased	Increased	No change
TNF α						No change	No change	No change
Leukocyte count	Kim, 2011[50]	1586	Asymptomatic, 20–86 years, mean 53.6	Whole blood	S. Korea	Increased	n/t	n/t

^aonly for periventricular hyperintensity, not for deep-and-subcortical white matter hyperintensity

^bin univariate analysis; association disappeared after adjustment for leukocyte count, age, sex, cardiovascular risk factors and aspirin use

^conly in black patients for WML and Hispanic patients for silent brain infarcts

CVD: cardiovascular disease (coronary heart disease or heart failure) n/t: not tested TNF: tumor necrosis factor WML: white matter lesion

Blood biomarkers for systemic inflammation, endothelial dysfunction and oxidative stress have been explored in relation to subclinical brain damage in the general population, in hypertensive patients and those with diabetes mellitus, but biomarkers more specific to brain damage have been less thoroughly investigated. Abraham's 2016 review of cardiovascular risk factors and small vessel brain disease, assessing studies associating nonglycemic blood biomarkers with WML, did not look at blood biomarkers specific to brain damage.[56]

Blood-based biomarkers specific to brain damage have been employed to assess degree of brain injury and as outcome predictors in different clinical contexts (stroke, traumatic brain injury and cardiac arrest, among others),[57–62] and also as end points for evaluating the effect of new therapies.[63] In general, notwithstanding the etiology of most brain lesions, at some point the blood–brain barrier becomes compromised, making it easier for cytosolic contents released from injured brain tissue to reach the

blood. Among these biomarkers, brain-specific proteins (neuronal or glial in origin) have received growing attention in clinical neurological research: neuron-specific enolase (NSE), a cytosolic neuronal protein;[57,59] S100B calcium-binding protein and glial fibrillary acidic protein (GFAP), both glial-derived proteins.[59–61] Also autoantibodies to brain-specific proteins have been the object of growing interest.[64] Studies employing autoantibodies to NR1[63] and NR2 peptides of NMDA receptors[65–68] have shown promise as biomarkers of brain damage in ischemic stroke.

The idea of employing blood-based biomarkers of brain damage for predicting subclinical brain damage in HT is attractive and is supported by the previously mentioned findings clearly demonstrating subclinical brain lesions in neurologically asymptomatic HT patients. These chronically affected brain regions—mainly involving white matter—could be leaking brain-specific molecules into the blood stream at a rate much lower than in acute brain injuries (stroke, brain trauma, global ischemia due to cardiac arrest). Nevertheless, the possibility of detecting these brain-specific proteins in blood is undoubtedly increasing with the greater availability and sensitivity of current commercial kits. In this respect, measurement of autoantibodies to these proteins or their subunits is even more promising when dealing with chronically established brain lesions, as occurs with HT.[64–69]

Some studies have examined serum concentrations of brain-specific proteins in asymptomatic HT persons, but without looking for associations with subclinical brain MRI changes. Dambinova reported no changes in NR2A autoantibodies in a young cohort of 25 HT patients (mean age 36.9 years).[66] Schmidt provided preliminary evidence that increased S100B was associated with eclampsia, but not with preeclampsia or chronic HT in pregnant women (although these conditions are very dissimilar to essential HT), hypothesizing that increased S100B could be secondary to cerebral vascular changes leading to overperfusion, edema and ischemia, as well as to seizures themselves.[70] Al-Rawi measured salivary and serum levels of NSE in 25 HT patients, observing higher mean serum NSE levels in HT patients than in healthy controls, with values ranging between those obtained in ischemic stroke patients and healthy controls. Differences were not statistically significant,[71] possibly because of small sample size.

Gruden studied 72 healthy volunteers of both sexes and different ages and blood pressure levels, measuring S100B, the HLDF24 peptide fragment of leukemia differentiation factor (HLDF) and idiotypic autoantibodies to these proteins to determine their possible usefulness as biomarkers for HT risk. They found that serum concentrations of these molecular factors varied significantly by blood pressure level, even between those with optimal blood pressure and those whose blood pressure was merely “normal.” The authors concluded that variations in serum concentration of these factors and their antibodies could reliably predict HT risk in healthy adults.[69]

Our review yielded that the only attempt to predict silent brain damage in essential HT by measuring brain-specific proteins in serum was a pilot study conducted by our group, where NSE and S100B serum concentrations were assessed in 50 asymptomatic essential HT patients. Serum levels of NSE and S100B were increased in HT patients, but only NSE levels were associated with indicators of HT severity (high diastolic blood pressure and retinopathy grade). Brain MRI conducted in 23 patients showed an association of higher NSE levels with more severe WML.[18] Increasing the sample size to 101 HT patients (34 with brain MRI

scans) produced similar findings. Thus, serum NSE levels could be useful in identifying HT patients with putative subclinical brain damage for subsequent brain MRI scanning. Longitudinal study of this cohort provided additional information suggesting that increased NSE could be a useful prognostic marker for central nervous system vascular events in asymptomatic HT patients.[72]

These results could constitute a baseline for future research on serum biomarkers for detection of subclinical brain damage in HT. Other molecules should be investigated, among which autoantibodies against NR1 or NR2 NMDA receptor peptides show promise, based on results obtained in studies of ischemic stroke.[65–68] These peptides also have the advantage of being very specific to the central nervous system, thus minimizing or eliminating the possibility of extracerebral sources.

Cuban experience According to Cuba’s 2012 Third National Survey on Risk Factors and Chronic Diseases, HT prevalence is 30.9% in the population aged ≥ 15 years. Treated HT patients comprise 89.2% of the total, but only 55.1% were reported as controlled.[73] Thus, essential HT and its target-organ sequelae constitute a major health problem in Cuba. Prevention is a fundamental principle of Cuba’s National Health System, so the 2008 Guidelines for Prevention, Diagnosis and Treatment of Arterial Hypertension emphasize not only HT prevention (the most important, universal and least expensive health intervention), but also preventing cardiovascular, cerebrovascular and renal diseases through identification of subclinical target organ damage.[74]

As we stated above, the frequency of subclinical brain damage in HT patients in the two Cuban research cohorts is comparable to that reported by other groups: 70.6% for a cohort with a mean age of 59 years[19] and 40% for a younger cohort (mean age: 44.2 years).[17] As in most countries, cardiorenal damage is routinely screened in HT patients, but low availability and high costs of brain MRI scanning preclude its use for early detection of asymptomatic brain lesions. Developing countries urgently need effective, less expensive and more accessible methods for widespread screening for subclinical brain damage, enabling most efficient use of scarce MRI resources.


Two Cuban research groups have focused on this goal. One group, at the Cuban Neuroscience Center, published the previously mentioned pilot study, reporting that quantitative EEG predicted subclinical brain damage on MRI much better than a battery of alternative tests (visual EEG, ABPM, fundoscopy and other clinical measures).[17] The results of another study involving the same patients (cited above) suggested that grand total EEG score could be used to screen for subclinical cerebrovascular lesions among HT patients, since it was associated with classical brain MRI lesions.[38]

The second research group, our own at the Cuban Neurology and Neurosurgery Institute, published a study in 50 patients with essential HT referred by family physicians for assessment of retinopathy.[18] Our main goal was to explore whether serum concentrations of brain-specific proteins (S100B and NSE) could predict subclinical brain MRI lesions. The results (summarized briefly above) indicated that higher serum NSE levels were useful predictors of more severe brain lesions in this group of patients. Similar results were observed when we later followed a larger cohort (101 HT patients) for an average of 33 months.[72] Our review did not find any earlier research[18] employing serum levels of brain-spe-

cific proteins to predict subclinical brain damage in HT patients. Finally, our group also reported a resistive carotid flow pattern in HT patients with classical MRI lesions, characterized by increased resistive index and decreased diastolic velocity, suggesting the potential usefulness of carotid structure and hemodynamics as imaging biomarkers of subclinical brain lesions.[19]

CONCLUSIONS

Prediction of subclinical brain damage in HT patients and other risk conditions for cerebrovascular disease has been explored

employing several biomarkers. To date, none of these has been introduced in general medical practice due mainly to prohibitive cost, and the need for specific technologies and highly specialized expertise. Although still infrequently assessed, blood-based biomarkers, especially brain-specific proteins, show promise as more accessible and less expensive tools to help stem the increased disease burden of stroke, cognitive decline and dementia. If proven effective, such biomarkers could have encouraging implications for resource-scarce settings, enabling countries such as Cuba to optimize use of MRI scanning. 

REFERENCES

- Mancia G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC Guidelines for the management of arterial HT: the task force for the management of arterial HT of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens*. 2013 Jul;31(7):1281–357.
- Maillard P, Seshadri S, Beiser A, Himali J, Au R, Fletcher E, et al. Effects of systolic blood pressure on white-matter integrity in young adults in the Framingham Heart Study: a cross-sectional study. *Lancet Neurol*. 2012 Dec;11(12):1039–47.
- Huang Y, Cai X, Li Y, Su L, Mai W, Wang S, et al. Prehypertension and the risk of stroke: a meta-analysis. *Neurology*. 2014 Apr 1;82(13):1153–61.
- Rosignol P, Hosseini K, Tropeano AI, Faya R, Tsatsaris A, Guillemin F, et al. Target organ damage assessment in French hypertensive patients without established cardiovascular or renal disease: results of the PREVENT-A study. *J Hypertens*. 2013 Jan;31(1):177–85.
- Mancia G, Laurent S, Agabiti-Rosei E, Ambrosioni E, Burnier M, Caulfield MJ, et al. European Society of Hypertension. Reappraisal of European guidelines on hypertension management: a European Society of Hypertension Task Force document. *J Hypertens*. 2009 Nov;27(11):2121–58.
- Vermeer SE, Longstreth WT Jr, Koudstaal PJ. Silent brain infarcts: a systematic review. *Lancet Neurol*. 2007 Jul;6(7):611–9.
- Henskens LH, van Oostenbrugge RJ, Kroon AA, Hofman PA, Lodder J, de Leeuw PW. Detection of silent cerebrovascular disease refines risk stratification of hypertensive patients. *J Hypertens*. 2009 Apr;27(4):846–53.
- Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol*. 2010 Jul;9(7):689–701.
- Scuteri A, Nilsson PM, Tzourio C, Redon J, Laurent S. Microvascular brain damage with aging and hypertension: pathophysiological consideration and clinical implications. *J Hypertens*. 2011 Aug;29(8):1469–77.
- Bernick C, Kuller L, Dulberg C, Longstreth WT Jr, Manolio T, Beauchamp N, et al. Silent MRI infarcts and the risk of future stroke: the cardiovascular health study. *Neurology*. 2001 Oct 9;57(7):1222–9.
- Vermeer SE, Hollander M, van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the Rotterdam Scan Study. *Stroke*. 2003 May;34(5):1126–9.
- Longstreth WT, Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, et al. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. *Stroke*. 1996 Aug;27(8):1274–82.
- Sierra C. Essential hypertension, cerebral white matter pathology and ischemic stroke. *Curr Med Chem*. 2014;21(19):2156–64.
- Liao D, Cooper L, Cai J, Toole JF, Bryan NR, Hutchinson RG, et al. Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control. The ARIC Study. *Stroke*. 1996 Dec;27(12):2262–70.
- Longstreth WT Jr, Bernick C, Manolio TA, Bryan N, Jungreis CA, Price TR. Lacunar infarcts defined by magnetic resonance imaging of 3660 elderly people: the Cardiovascular Health Study. *Arch Neurol*. 1998 Sep;55(9):1217–25.
- van Boxtel M, Henskens LH, Kroon AA, Hofman PA, Gronenschild EH, Jolles J, et al. Ambulatory blood pressure, asymptomatic cerebrovascular damage and cognitive function in essential hypertension. *J Hum Hypertens*. 2006 Jan;20(1):5–13.
- Hernández-González G, Bringas-Vega ML, Galán-García L, Bosch-Bayard J, Lorenzo-Ceballos Y, Melie García L, et al. Multimodal quantitative neuroimaging databases and methods: the Cuban Brain Mapping Project. *Clin EEG Neurosci*. 2011 Jul;42(3):149–59.
- González-Quevedo A, García SG, Concepción OF, Freixas RS, Quevedo Sotolongo LQ, Menéndez MC, et al. Increased serum S-100B and neuron specific enolase—potential markers of early nervous system involvement in essential hypertension. *Clin Biochem*. 2011 Feb;44(2–3):154–9.
- González-García S, Hernández-Díaz Z, Quevedo-Sotolongo L, Peña-Sánchez M, Pino-Peña Y, Fernández-Carriera R, et al. Resistive cerebral blood flow as a potential marker of subclinical brain damage in essential hypertension. *World J Cardiovasc Dis*. 2014;4(4):169–78.
- Van Dijk EJ, Prins ND, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MM. Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam Scan study. *Stroke*. 2008 Oct;39(10):2712–9.
- Gottesman RF, Coresh J, Catellier DJ, Sharrett AR, Rose KM, Coker LH, et al. Blood pressure and white-matter disease progression in a biethnic cohort: Atherosclerosis Risk in Communities (ARIC) study. *Stroke*. 2010 Jan;41(1):3–8.
- Sierra C. Associations between ambulatory blood pressure parameters and cerebral white matter lesions. *Int J Hypertens*. 2011;2011:478710. DOI: 10.4061/2011/478710.
- De Groot JC, de Leeuw FE, Oudkerk M, van Gijn J, Hofman A, Jolles J, et al. Cerebral WML and cognitive function: the Rotterdam Scan Study. *Ann Neurol*. 2000;47:145–51.
- Prabhakaran S, Wright CB, Yoshita M, Delapaz R, Brown T, DeCarli C, et al. Prevalence and determinants of subclinical brain infarction: the Northern Manhattan Study. *Neurology*. 2008 Feb 5;70(6):425–30.
- Wright CB, Festa JR, Paik MC, Schmiedigen A, Brown TR, Yoshita M, et al. White matter hyperintensities and subclinical infarction: associations with psychomotor speed and cognitive flexibility. *Stroke*. 2008 Mar;39(3):800–5.
- Delgado P, Riba-Llena I, Tovar JL, Jarca CI, Mundet X, López-Rueda A, et al. Prevalence and associated factors of silent brain infarcts in a Mediterranean cohort of hypertensives. *Hypertension*. 2014 Sep;64(3):658–63.
- Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, et al. An updated definition of stroke for the 21st century: a statement for health care professionals from the American Heart Association/American Stroke Association. *Stroke*. 2013 Jul;44(7):2064–89.
- Kitagawa K. Cerebral blood flow measurement by PET in hypertensive subjects as a marker of cognitive decline. *J Alzheimers Dis*. 2010;20(3):855–9.
- Dijkhuizen RM. Advances in MRI-based detection of cerebrovascular changes after experimental traumatic brain injury. *Transl Stroke Res*. 2011 Dec;2(4):524–32.
- Cheung CY, Tay WT, Mitchell P, Wang JJ, Hsu W, Lee ML, et al. Quantitative and qualitative retinal microvascular characteristics and blood pressure. *J Hypertens*. 2011 Jul;29(7):1380–91.
- Grassi G, Schmedier RE. The renaissance of the retinal microvascular network assessment in hypertension: new challenges. *J Hypertens*. 2011 Jul;29(7):1289–91.
- De Quesada-Martínez M, Blanco-García M, Díaz-Quesada L. Alteraciones funcionales cerebrales tempranas en pacientes con hipertensión arterial no complicada. *Rev Neurol*. 2005 Feb 16;28(4):199–209. Spanish.
- Goldstein B, Bartzokis G, Guthrie D, Shapiro D. Ambulatory blood pressure and the brain: a 5-year follow-up. *Neurology*. 2005 Jun 14;64(11):1846–52.
- Avet J, Pichot V, Barthélémy JC, Laurent B, Garcin A, Roche F, et al. Leukoaraiosis and ambulatory blood pressure load in a healthy elderly cohort study: the PROOF study. *Int J Cardiol*. 2014 Mar 1;172(1):59–63.
- Scheie HG. Evaluation of ophthalmoscopic changes of hypertension and arteriolar sclerosis. *AMA Arch Ophthalmol*. 1953 Feb;49(2):117–38.
- Wong TY, Klein R, Sharrett AR, Couper DJ, Klein BE, Liao DP, et al. Cerebral white matter lesions, retinopathy, and incident clinical stroke. *JAMA*. 2002 Jul;288(1):67–74.
- De Quesada Martínez ME, Reyes Moreno M. Localización de la actividad paroxística en pacientes con hipertensión arterial con el uso de la tomografía electromagnética de baja resolución (LORETA). *Vitae Acad Bioméd Digital [Internet]*. 2010 [cited 2015 Apr 17];44. Available from: <http://dialnet.unirioja.es/servlet/articulo?codigo=3749913>. Spanish.
- Brown Martínez M, Valdés-González Y, González-Ortiz E, Hernández-González G, Valdés Sosa P, Galán García L, et al. [Use of electroencephalography to identify asymptomatic cerebrovascular lesions among hypertensives]. *Rev Cub Invest Bioméd*. 2014;33:231–40. Spanish.
- Kearney-Schwartz A, Rosignol P, Bracard S, Felblinger J, Fay R, Boivin JM, et al. Vascular structure and function is correlated to cognitive perfor-

- mance and white matter hyperintensities in older hypertensive patients with subjective memory complaints. *Stroke*. 2009 Apr;40(4):1229–36.
40. Heliopoulos I, Artemis D, Vadikolias K, Tripsianis G, Piperidou C, Tsvigoulis G. Association of ultrasonographic parameters with subclinical white-matter hyperintensities in hypertensive patients. *Cardiovasc Psychiatry Neurol*. 2012; 2012:616572. DOI: 10.1155/2012/616572.
 41. Scuteri A. Brain injury as end-organ damage in hypertension. *Lancet Neurol*. 2012 Dec;11(12):1015–7.
 42. Gaşeci D, Kwarciany M, Nyka W, Narkiewicz K. Hypertension, brain damage and cognitive decline. *Curr Hypertens Rep*. 2013 Dec;15(6):547–58.
 43. Emerging Risk Factors Collaboration, Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet*. 2010 Jan 9;375(9709):132–40.
 44. Jiménez MC, Rexrode KM, Glynn RJ, Ridker PM, Gaziano JM, Sesso HD. Association between high-sensitivity C-reactive protein and total stroke by hypertensive status among men. *J Am Heart Assoc*. 2015 Sep 21;4(9):e002073. DOI: 10.1161/JAHA.115.002073.
 45. Wright CB, Moon Y, Paik MC, Brown TR, Rabhani L, Yoshita M, et al. Inflammatory biomarkers of vascular risk as correlates of leukoariosis. *Stroke*. 2009 Nov;40(11):3466–71.
 46. Satizabal CL, Zhu YC, Mazoyer B, Dufouil C, Tzourio C. Circulating IL-6 and CRP are associated with MRI findings in the elderly: the 3C-Dijon Study. *Neurology*. 2012 Mar 6;78(10):720–7. DOI: 10.1212/WNL.0b013e318248e50f. Epub 2012 Feb 22.
 47. Mitaki S, Nagai A, Oguro H, Yamaguchi S. C-reactive protein levels are associated with cerebral small vessel-related lesions. *Acta Neurol Scand*. 2015 May 14. DOI: 10.1111/ane.12440. [Epub ahead of print]
 48. Abe A, Nishiyama Y, Harada-Abe M, Okubo S, Ueda M, Mishina M, et al. Relative risk values of age, acrolein, IL-6 and CRP as markers of periventricular hyperintensities: a cross-sectional study. *BMJ Open*. 2014;4(8):e005598.
 49. Shoamanesh A, Preis SR, Beiser AS, Vasan RS, Benjamin EJ, Kase CS, et al. Inflammatory biomarkers, cerebral microbleeds, and small vessel disease. *Framingham Heart Study*. *Neurology*. 2015 Feb 24;84(8):825–32.
 50. Kim CK, Lee SH, Kim BJ, Ryu WS, Choi SH, Oh BH, et al. Elevated leukocyte count in asymptomatic subjects is associated with a higher risk for cerebral white matter lesions. *Clin Neurol Neurosurg*. 2011 Apr;113(3):177–80.
 51. Hudson BI, Moon YP, Kalea AZ, Khatri M, Marquez C, Schmidt AM, et al. Association of serum soluble Receptor for Advanced Glycation End-products with subclinical cerebrovascular disease: The Northern Manhattan Study (NOMAS). *Atherosclerosis*. 2011 May;216(1):192–8.
 52. Dadu RT, Fornage M, Virani SS, Nambi V, Hoogeveen RC, Boerwinkle E, et al. Cardiovascular biomarkers and subclinical brain disease in the atherosclerosis risk in communities study. *Stroke*. 2013 Jul;44(7):1803–8.
 53. Reinhard H, Garde E, Skimminge A, Åkeson P, Ramsøy TZ, Winther K, et al. Plasma NT-proBNP and white matter hyperintensities in type 2 diabetic patients. *Cardiovasc Diabetol*. 2012 Oct 3;11:119. DOI: 10.1186/1475-2840-11-119.
 54. de Leeuw FE, de Kleine M, Frijns CJ, Fijnheer R, van Gijn J, Kappelle LJ. Endothelial cell activation is associated with cerebral white matter lesions in patients with cerebrovascular disease. *Ann NY Acad Sci*. 2002 Nov;977:306–14.
 55. Markus HS, Hunt B, Palmer K, Enzinger C, Schmidt H, Schmidt R. Markers of endothelial and hemostatic activation and progression of cerebral white matter hyperintensities: longitudinal results of the Austrian Stroke prevention study. *Stroke*. 2005 Jul;36(7):1410–4.
 56. Abraham HM, Wolfson L, Moscufo N, Guttmann CR, Kaplan RF, White WB. Cardiovascular risk factors and small vessel disease of the brain: Blood pressure, white matter lesions, and functional decline in older persons. *J Cereb Blood Flow Metab*. 2016;36(1):132–42.
 57. Cheng F, Yuan Q, Yang J, Wang W, Liu H. The prognostic value of serum neuron-specific enolase in traumatic brain injury: systematic review and meta-analysis. *PLoS One*. 2014 Sep 4;9(9):e106680. DOI:10.1371/journal.pone.0106680.
 58. Di Pietro V, Amorini AM, Lazzarino G, Yakoub KM, D'Urso S, Lazzarino G, et al. S100B and glial fibrillary acidic protein as indexes to monitor damage severity in an in vitro model of traumatic brain injury. *Neurochem Res*. 2015 May;40(5):991–9.
 59. Shinozaki K, Oda S, Sadahiro T, Nakamura M, Hirayama Y, Abe R, et al. S-100B and neuron-specific enolase as predictors of neurological outcome in patients after cardiac arrest and return of spontaneous circulation: a systematic review. *Crit Care*. 2009;13(4):R121.
 60. Pearlman DM, Brown JR, MacKenzie TA, Hernandez F Jr, Najjar S. Blood levels of S-100 calcium-binding protein B, high-sensitivity C-reactive protein, and interleukin-6 for changes in depressive symptom severity after coronary artery bypass grafting: prospective cohort nested within a randomized, controlled trial. *PLoS One*. 2014 Oct 20;9(10):e111110. DOI:10.1371/journal.pone.0111110
 61. Strathmann FG, Schulte S, Goerl K, Petron DJ. Blood-based biomarkers for traumatic brain injury: evaluation of research approaches, available methods and potential utility from the clinician and clinical laboratory perspectives. *Clin Biochem*. 2014 Jul;47(10–11):876–88.
 62. Gazzolo D, Li Volti G, Gavilanes AW, Scapagnini G. Biomarkers of brain function and injury: biological and clinical significance. *Biomed Res Int* [Internet]. 2015 [cited 2015 Apr 17];2015:389023. Available from: <http://dx.doi.org/10.1155/2015/389023>
 63. Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M, et al. Erythropoietin therapy for acute stroke is both safe and beneficial. *Mol Med*. 2002 Aug;8(8):495–505.
 64. Dalmaj J. Name a brain protein, and an autoantibody shall be found! *Neurol Neuroimmunol Neuroinflamm*. 2015 Oct 8;2(5):e159. DOI: 10.1212/NXI.0000000000000159.
 65. Zerche M, Weissenborn K, Ott C, Dere E, Asif AR, Worthmann H, et al. Preexisting serum autoantibodies against the NMDAR subunit NR1 modulate evolution of lesion size in acute ischemic stroke. *Stroke*. 2015 May;46(5):1180–6.
 66. Dambinova SA, Kounteev GA, Skoromets AA. Multiple panel of biomarkers for TIA/stroke evaluation. *Stroke*. 2002 May;33(5):1180–1.
 67. Dambinova SA, Kounteev GA, Izykenova GA, Zavolokov IG, Ilyukhina AY, Skoromets AA. Blood test detecting autoantibodies to N-methyl-D-aspartate neuroreceptors for evaluation of patients with transient ischemic attack and stroke. *Clin Chem*. 2003 Oct;49(10):1752–62.
 68. Weissman JD, Khunteev GA, Heath R, Dambinova SA. NR2 antibodies: risk assessment of transient ischemic attack (TIA)/stroke in patients with history of isolated and multiple cerebrovascular events. *J Neurol Sci*. 2011 Jan 15;300(1–2):97–102.
 69. Gruden MA, Elistratova EI, Kudrina MV, Karolina VP, Deryabina IB, Semenova IM, et al. Content of S100b protein, HLDF24 peptide and autoantibodies to these factors as potential biomarkers for arterial hypertension in blood serum of healthy people. *Bull Exp Biol Med*. 2014 Feb;156(4):426–9.
 70. Schmidt AP, Tort AB, Amaral OB, Schmidt AP, Walz R, Vettorazzi-Stuckzynski J, et al. Serum S100B in pregnancy-related hypertensive disorders: a case-control study. *Clin Chem*. 2004 Feb;50(2):435–8.
 71. Al-Rawi NH, Atiyah KM. Salivary neuron specific enolase: an indicator for neuronal damage in patients with ischemic stroke and stroke-prone patients. *Clin Chem Lab Med*. 2009;47(12):1519–24.
 72. González-Quevedo A, González-García S, Hernández-Díaz Z, Fernández-Concepción O, Quevedo-Sotolongo L, Peña-Sánchez M, et al. Serum neuron specific enolase could predict subclinical brain damage and the subsequent occurrence of brain related vascular events during follow up in essential hypertension. *J Neurol Sci*. 2016;363:158–163.
 73. Bonet Gorbea M, Varona Pérez P. III Encuesta Nacional de factores de riesgo y actividades preventivas de enfermedades no transmisibles. Cuba 2010–2011 [Internet]. Havana: Editorial Ciencias Médicas; 2015 [cited 2015 Apr 13]. p. 140–65. Available from: http://www.bvs.sld.cu/libros/encuesta_nacional Riesgo/hipertension.pdf. Spanish.
 74. Pérez Caballero MD, Dueñas Herrera A, Alfonso Guerra JP, National Technical Committee for the Arterial Hypertension Program. *Hipertensión arterial. Guía para la prevención, diagnóstico y tratamiento*. Havana: Editorial Ciencias Médicas; 2008. Spanish.

THE AUTHORS

Alina González-Quevedo (Corresponding author: aglez@infomed.sld.cu), physician. Senior researcher, full professor of biochemistry at the Cuban Neurology and Neurosurgery Institute (INN) and Medical University of Havana (UCMH), Cuba.

Sergio González-García, biochemist. Senior researcher, full professor of biochemistry, INN, UCMH, Havana, Cuba.

Marisol Peña-Sánchez, biochemist. Assistant researcher, assistant professor of biochemistry, INN, UCMH, Havana, Cuba.

Caridad Menéndez-Sainz, biochemist. Senior researcher, full professor of biochemistry, INN, UCMH, Havana, Cuba.

Rebeca Fernández-Carriera, biochemist. Assistant researcher, assistant professor of biochemistry, INN, UCMH, Havana, Cuba.

Anay Cordero-Einz, pharmacist. Assistant researcher and professor of pharmacology, INN, UCMH, Havana, Cuba.

Submitted: September 2, 2015

Approved for publication: April 17, 2016

Disclosures: None