Minireview

How S100B crosses brain barriers and why it is considered a peripheral marker of brain injury

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Impact statement

S100B is a calcium-binding protein that we have been working on for the last 20 years, which is widely used as a marker of brain damage, experimentally and clinically, including now during the SARS-CoV-2 pandemic. We discuss the concept of S100B as an alarmin and its dual activity as an inflammatory and neurotrophic molecule. We discuss the contribution of extracerebral sources to serum levels of S100B. Furthermore, we emphasize the lack of data supporting the idea that S100B acts as a marker of blood–brain barrier rupture, and the need to include the glymphatic system in the interpretations of serum changes of S100B.

Abstract

S100B is a 21-kDa protein that is produced and secreted by astrocytes and widely used as a marker of brain injury in clinical and experimental studies. The majority of these studies are based on measurements in blood serum, *assuming* an associated increase in cerebrospinal fluid and a rupture of the blood–brain barrier (BBB). Moreover, extracerebral sources of S100B are often underestimated. Herein, we will review these interpretations and discuss the routes by which S100B, produced by astrocytes, reaches the circulatory system. We discuss the concept of S100B as an alarmin and its dual activity as an inflammatory and neurotrophic molecule. Furthermore, we emphasize the lack of data supporting the idea that S100B acts as a marker of BBB rupture, and the need to include the glymphatic system in the interpretations of serum changes of S100B. The review is also dedicated to valorizing extracerebral sources of S100B, particularly adipocytes. Furthermore, S100B *per se* may have direct and indirect modulating roles in brain barriers: on the tight junctions that regulate paracellular transport; on the expression of its receptor, RAGE, which is involved in transcellular protein transport; and on aquaporin-4, a key protein in the glymphatic system that is responsible for the clearance of extracellular proteins from the central nervous system. We hope that the data on S100B, discussed here, will be useful and that it will translate into further health benefits in medical practice.

Keywords: Astrocyte, AQP-4, BBB, glymphatic system, RAGE, S100B, tight junctions

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Introduction

S100B is a small soluble protein that is widely used as a marker of brain injury in clinical and experimental studies. In brain tissue, it is predominantly derived from astrocytes, and it is possible to detect it in cerebrospinal fluid (CSF), blood, and urine samples. Increases in S100B in the serum or urine are commonly interpreted as secondary to an increase in the CSF due to astroglial activation and/or rupture of the blood–brain barrier (BBB). However, the mechanism of release of glial S100B and its passage into the blood is debatable. Herein, we will review these interpretations and discuss the routes of how S100B, produced by astrocytes, reaches the circulatory system.

The vast majority of studies of S100B as a marker of brain injury are based on measurements in the blood serum, *presuming* an associated increase in CSF. To evaluate the relationship between CSF and serum S100B, we performed a preliminary systematic review search on PubMed, with the words S-100 OR S100 AND CSF AND serum. We found 153 references (including 12 reviews) (Figure 1), where increased serum S100B is commonly associated with an increase in

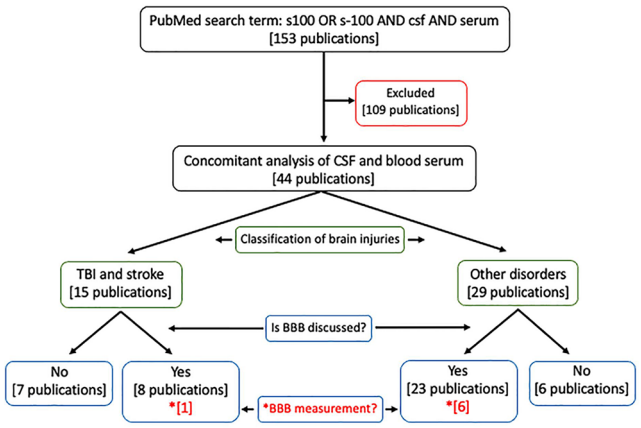

Figure 1. Flowchart of selected studies. A preliminary sample on the subject was made with a selection of articles in PubMed, on 9 May 2023, using the terms: S100 OR S-100 AND serum AND CSF. Of the 153 articles, 109 were excluded because they did not involve concomitant measurement of S100B in blood serum and CFS. The number of articles selected is in square brackets, at different stages of classification.

brain tissue and/or a disruption of the BBB. However, the origin and flow of S100B are complex events and require caution in their interpretation.

What is the S100B protein?

S100B means S100 calcium-binding protein B, according to the official nomenclature.[1] Other less commonly used names include S-100B, S100β, and neurite extension factor (NEF). This 21-kDa protein has a homodimeric organization (two beta subunits), where each subunit has two EF-hand calcium-binding sites and independent zinc-binding sites. The S100 family of calcium-binding proteins (which contains dozens of members) is characterized by high solubility (even in sodium persulfate at 100%). In the nervous system, S100B is mainly expressed in astrocytes but also in oligodendrocytes, Schwann cells, and epithelial cells of the choroid plexus. Our group has investigated the secretion of S100B by astrocytes, in cell cultures and brain slices, and peripheral S100B in patients and disease models with nervous system damage, although the mechanism(s) of secretion, by a non-classical and Ca^{2+}-dependent pathway remains unclear.[2–5]

S100B has numerous intracellular targets/partners, including cytoskeletal proteins (e.g. glial fibrillary acidic protein [GFAP]), calcium signaling proteins (e.g. reticular Ca^{2+} channels, AHNAK, and calcineurin), nuclear signaling proteins (e.g. p53 and Ndr kinase), and enzymes of glucose metabolism (e.g. phosphoglucomutase). It is not our intention to discuss the intracellular role of S100B (see Donato *et al.*[6] and Baudier and Gentil[7] for a review) nor the causal relationship of the protein with neurological or psychiatric diseases (see Schroeter *et al.*[8] and Michetti *et al.*[9] for a review). For evaluating the extracellular role of S100B, even as a marker of brain damage, we will mention its best characterized extracellular target, the RAGE (receptor for advanced glycated end products). However, just like S100B has many intracellular targets, acting as a polyvalent chaperone/modulator, it potentially has many emerging extracellular targets in addition to RAGE, such as β-amyloid peptides,[10] calcium- and potassium-channels,[11] which we will not discuss here.

Why is S100B considered a brain injury marker?

In the literature, there are now about 40 meta-analyses relating serum S100B to a variety of neurological and psychiatric diseases, mainly traumatic brain injury (TBI) and stroke, but associations with many other disorders have been reported, including Alzheimer's disease, multiple sclerosis, schizophrenia, bipolar disorder, and major depression. There is no doubt that the increase in S100B in the CSF or serum can be used as a marker of brain injury to assess severity, prognosis, and even monitoring of treatment, although the causal relationship with these neuropsychiatric disorders is debatable.[8,9]

In the innate immune response, two components are initially activated. These components are distinct, but they share many features: (1) the danger signals (which come from a pathogen aggressor and are, therefore, called pathogen-associated molecular patterns [PAMPs], or come from the attacked cells, and are called danger-associated molecular patterns [DAMPs] or simply alarmins); and (2) cytokines, which are the proteins that are synthesized in response to the former. We are not going to discuss concepts and examples here, which would require a separate review. S100B, as it belongs to the S100 family, is considered an alarmin[12] and indeed, many metabolic (e.g. ischemia) and inflammatory (e.g. lipopolysaccharide [LPS]) dysfunctions[3,13] induce the release of S100B. This protein can act as a danger signaling protein, which acts on RAGE (and other extracellular targets) at nM levels, promoting defense, recovery, and even proliferation of neural cells, but results, *in vitro*, also indicate that, at high concentrations (mM), S100B can cause neuronal death.[6]

However, S100B differs from other alarmin proteins, such as high mobility group box-1 (HMGB1) and heat shock protein 70 (HSP-70), which are ubiquitous. We thus may consider S100B to be a cell-specific alarmin, that is, astrocyte-specific. In this way, all other members of the family are cell-specific alarmins according to the cells where they are expressed, although not all members of the family are alarmins. Due to its trophic effects (including neurite extension), some authors also consider S100B to be a neurotrophic cytokine.[14] In adipose tissue (discussed next), S100B has also been suggested to act as a cytokine, more specifically an adipokine.[15,16] Indeed, S100B both induces and is induced by other cytokines, sharing many inflammatory cytokine-signaling pathways, including the nuclear factor-κB (NF-kB) and Janus kinases/Signal transducer and activator of transcriptions (JAK-STAT) pathways. However, whether an alarmin or cytokine, and even independently of this, the increase in S100B, despite its limitations, can be very useful as a marker of brain injury.

Serum S100B comes from cerebral and extracerebral sources

In the central nervous system (CNS), S100B is predominantly expressed and actively secreted by astrocytes,[17] but

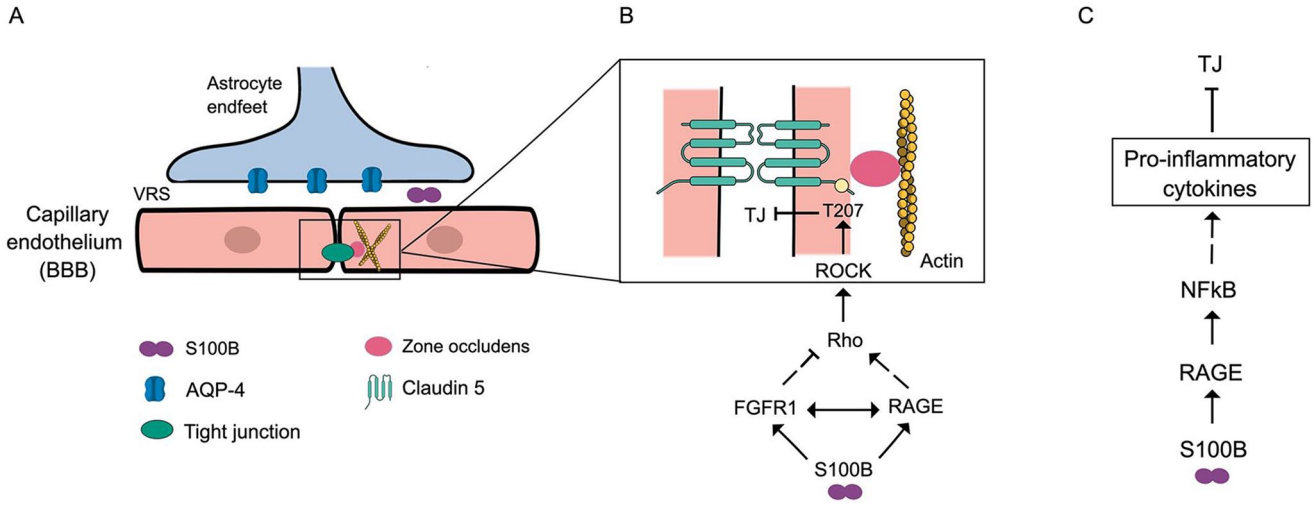

Figure 2. Structural organization of the BBB, with emphasis on claudin-5 from the TJ. (a) Representation of the endothelium of cerebral capillaries (BBB), with a more apical TJ formed by claudin-5, ZO-1 protein, and actin microfilaments. Surrounding the capillary is an endfoot astrocyte, containing aquaporin-4 (AQP-4). S100B is represented in the space between the glial cell and the endothelium (VRS, Virchow–Robin space); (b) The most detailed representation of the TJ, showing the bridge between the claudins and highlighting the intracellular threonine residue 207 of claudin-5, whose phosphorylation by Rho kinase (ROCK) facilitates the opening of the TJ. The regulatory relationships of extracellular S100B with the RAGE and FGFR1 receptors that modulate the downstream Rho protein, which, in turn, activate ROCK, are detailed in the text; (c) Representation of TJ destabilization by inflammatory cytokines, induced by S100B via the RAGE-NFkB pathway.

oligodendrocytes and some cholinergic neurons also express this protein. Many other cells, especially neural crest derivatives, express S100B, such as Schwann cells, enteric glial cells, glomus cells (from respiratory chemosensors), and chromaffin cells, but also some non-neural cells, such as adipocytes, melanocytes, muscle satellite cells, chondrocytes, lymphocytes, and dendritic cells, and some tumor cells, injured cardiomyocytes, and even activated macrophages also express S100B.[6,18] Of these extracerebral sources, it is worth emphasizing the adipocytes – which, due to the high expression and distribution of the adipose tissue, actively contribute to the serum content of S100B,[7,18–20] in addition to chondrocytes, melanocytes (not only in cases of melanoma) and, more recently, enteric glia.[21]

Is S100B able to cross the BBB?

The BBB is the most extensive of the brain barriers,[22] but other barriers, such as the blood–CSF barrier (BCSFB) in the choroid plexuses and the arachnoid brain barrier (ABB) in the arachnoid meninges should not be overlooked, except for didactic purposes, as we will do here in this section. However, these barriers provide an idea of the origin and flow of brain fluids only when contemplated together. Furthermore, the glymphatic system, as we call it today, affords an important mechanism for the clearance of brain extracellular proteins, including S100B, as we will discuss in section "The glymphatic system transports S100B from the brain interstitial fluid to the CSF and then to the bloodstream."

To reach CNS cells, a substance must cross the BBB, which is basically composed of endothelial cells, and distinct from other regions due to the lack of fenestrations and the presence of tight junctions (TJ) that limit (practically prevent) paracellular transport. Furthermore, the BBB has selective membrane receptors, which are responsible for the transcellular transport of specific metabolites and proteins and prevent the entry of almost 99% of circulating xenobiotics.[23] Later, we will discuss the possibility that transcellular passage of S100B occurs. However, in this section, we will discuss paracellular transport.

Although there are other junctions between brain endothelial cells, the TJ are the most apical and are responsible, in the most part, for paracellular "impermeability."[24] The main TJ proteins are claudins, occludins, and junctional adhesion molecules (JAM). These integral membrane proteins interact with each other to make intercellular bridges and connect to scaffolding cytoskeletal proteins called ZO (from *zona occludens*), which, in turn, anchor to actin microfilaments (Figure 2). Claudins are the most numerous members (27 members) and are subject to numerous posttranslational changes, making them fundamental elements in the complexity and dynamics of TJ.[25]

Claudin-5 is predominantly encountered in the brain endothelium and, therefore, has been used as a pharmacological target to selectively open the BBB.[26] Phosphorylation of this protein at the intracellular site of threonine 207 by protein kinase A (PKA) or Rho kinase (ROCK) could facilitate the opening of the BBB. However, claudin-2 is not expressed in the BBB. This claudin, unlike the others, works as a channel for water and cations[27] and is localized in the TJ of choroid plexus epithelial cells and arachnoid cells,[28] indicating that under physiological conditions, not even water enters or leaves the BBB paracellular pathway. With the temporary rupture of the BBB, through a hyperosmotic shock with mannitol (whose mechanism remains unknown), the BBB opens pores of 20 nm, allowing the passage of large proteins, such as albumin (66 kDa, with a diameter of 3.8 nm). Under these conditions, S100B could easily pass, obeying a diffusion gradient, from the inside to the outside, but needing to overcome the hydrostatic pressure difference between the blood and the brain interstitial fluid.

TJ opening, often used as a synonym for BBB rupture, occurs in acute conditions associated with brain damage, such as stroke and TBI. In these cases, we can didactically divide the damage into two biochemical phases, which can overlap, to explain the opening of the TJ. The first phase (excitotoxic phase) occurs as a result of energy failure due to isolation (caused by the formation of a thrombus, clot, or edema), which results in an overload of Ca^{2+} in neurons, release of glutamate and glutamatergic overactivation. In this phase, alarmins are released, which initiate the second phase (inflammatory phase). Both alarmins and local oxidative stress, at this stage, induce TJ disruption through the posttranslational changes and protein oxidation that they cause, respectively. In the neuroinflammatory phase, the mechanisms also involve signaling alterations and post-translational changes in TJ proteins, mediated by cytokines, chemokines, metalloproteases, and oxidative stress.[29] Thus, under these conditions, extracellular S100B (increased or not) could flow into the blood and reflect brain and BBB damage.

Any inflammatory process in the CNS, either acute (e.g. due to COVID-19 or exacerbation of systemic lupus erythematosus) or chronic (e.g. due to diabetes mellitus or Alzheimer's disease) can result in the opening of the BBB, through cytokinergic activation (Figure 2(c)). Proinflammatory cytokines (IL-1β, TNF-α, and IL-6) destabilize TJ, while anti-inflammatory cytokines (e.g. IL-10) stabilize them. It should be mentioned that data from cell cultures show that S100B stimulates the release of proinflammatory cytokines and vice versa.[30,31]

Could the alarmin/cytokine role of S100B per se modulate BBB permeability?

Astrocytes are the most abundant and heterogeneous glial cells. They interact with microglia, envelop synapses and brain vessels, and form extensive astroglial networks through gap junctions. Pericapillary astrocytes secrete glial-derived neutrophic factor (GDNF) and basic fibroblast growth factor (bFGF), which stabilize TJ. In pathological situations, reactive astrocytes secrete mediators that destabilize TJ, such as proinflammatory cytokines, chemokines (which attract and modulate macrophage diapedesis), and nitric oxide.[32] In response to various stimuli, S100B is secreted by astrocytes[6] into the interstitial fluid, from where it diffuses easily due to its size and solubility and the lack of TJ among astrocytes. However, for the same reason, it does not cross the BBB. From the brain interstitial fluid, S100B (like other proteins) will drain into the CSF (see section "The glymphatic system transports S100B from the brain interstitial fluid to the CSF and then to the bloodstream").

Extracellular S100B binds to the RAGE, a multiligand receptor of the immunoglobulin superfamily.[6] Other RAGE ligands include advanced glycation end products (AGEs), β-amyloid peptides, and HMGB1 protein, a classical alarmin. Brain endothelial cells express RAGE and this receptor is involved in the transcellular transport of β-amyloid peptide and S100B across the BBB (as will be discussed in section "The putative transcellular transport of S100B in the brain barriers via RAGE"). RAGE activation triggers downstream molecules, such as p38-MAPK and calcineurin, which activate inflammatory pathways, such as NFAT and NF-kB, and result in the upregulation of cytokines, metalloproteases, and RAGE itself, and the downregulation of TJ proteins, resulting in the opening of the BBB.[33,34] Therefore, S100B could contribute to the opening of the BBB via cytokine upregulation. Furthermore, transduction mechanisms associated with RAGE suggest that S100B can directly modulate TJ, through claudin-5 phosphorylation,[23] via Rho protein activation and consequent ROCK activation[35] (Figure 2(b)). That is, RAGE activation could result in TJ destabilization. However, based on results with cultured myoblasts,[36] S100B could also bind bFGF and activate the FGF receptor 1 (FGFR1), leading to the inhibition of Rho protein, which in the endothelium could result in TJ stabilization. In addition, RAGE and FGFR interact, indicating that, in certain situations, S100B could favor the opening or closing of the BBB.

Ischemia, excitotoxicity, and high potassium affect S100B secretion

In section "Is S100B able to cross the BBB?," we pointed out that, in cases of stroke and TBI, an excitotoxic phase (caused by increased glutamate release) precedes the inflammatory phase. In both phases, there is rupture of the BBB. S100B secretion is stimulated in brain slices that are subjected to glucose and O_2 deprivation, mimicking a situation of ischemia.[37] However, high concentrations of glutamate in cultured astrocyte cells and brain slices reduce the secretion of S100B, by a mechanism independent of glutamatergic receptors, but dependent on glutamate transport.[38,39] The entry of glutamate, under these conditions, favors the activation of the Krebs cycle by generating α-ketoglutarate, negatively modulating the release of S100B induced by ischemia.[40]

However, compounds such as streptozotocin (which alters glucose influx) and fluorocitrate (which blocks the Krebs cycle) decrease basal secretion of S100B in brain slices.[41,42] Together, these data indicate that there is a functional connection (still unclear) between glucose flux and S100B secretion. Moreover, basal S100B secretion from rat hippocampal slices is reduced in high-K^+ medium,[43] but not in slices from Li-pilocarpine-treated rats.[44] The increase in S100B secretion from astrocytes and brain slices may explain the increase in the protein in the CSF that is observed in models of ischemia, TBI, and epilepsy.[45,46] The increase in peripheral S100B in patients with ischemic strokes or TBI has been abundantly demonstrated, particularly in blood samples. Although there have been studies with CSF from patients, the vast majority of studies report serum measurements. However, in some situations, S100B blood measurements do not reflect variations in CSF, as will see in the next section.

However, it should first be discussed whether S100B in CSF adequately reflects brain parenchyma damage, considering that it is an alarmin. First, the CSF S100B is a weighted average of the amounts secreted from the different brain regions. Second, it is estimated that, constitutively, less than 1% of the intracellular content of S100B is secreted.[2] Third, we know that astrocytes that are remotely located from a given lesion also often react, although not necessarily in the same way due to phenotypic heterogeneity.[47,48] This means

that astrocytes in remote regions could double or halve their secretion of S100B, without this representing a significant intracellular change in these regions or having an important effect on the CSF. As such, it is possible that, in the case of S100B, the increase in CSF may not exclusively reflect what happens at the site of a lesion in the brain parenchyma.

However, S100B is more than an alarmin. It has a neurotrophic function and may be important, as a consequence, in neuronal survival and even hippocampal neurogenesis.[49–51] It is important to observe increases in S100B in the CSF carefully, assessing by *how much* and *when* S100B increases. We also need to consider that increases in S100B (a secretable protein with an extracellular function) in the CSF differ from increases in other proteins, such as GFAP (also from astrocytes) or neuron-specific enolase (NSE) from neurons. These proteins are released (not secreted) when there is a loss of cellular integrity. An increase in GFAP or NSE in the CSF reflects loss of glial or neuronal integrity, respectively, in some regions. In cases of massive "gliolysis," very high levels of S100B and GFAP are seen in patients, leading to contemplation of a clinically worse prognosis.[52,53] As such, two fractions of S100B may exist in the CSF, secreted and released S100B, which adds a degree of complexity to the interpretation of clinical and experimental data.

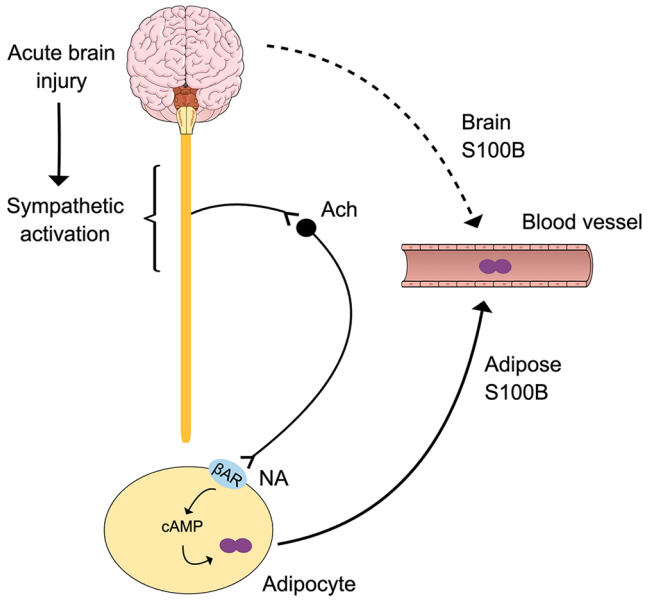

Figure 3. Increased serum S100B during severe and acute brain injury situations. In these situations (TBI or stroke), there is sympathetic hyperactivation. Cholinergic (ganglionic) and adrenergic (postganglionic) activation triggers the release of S100B from adipocytes, via beta-adrenergic receptors (βAR), and mediated by cAMP. A "slower" cerebral release of S100B, dependent on traffic in the glymphatic system, is represented by the dashed line.

Does the increase in S100B in the CSF imply an increase in S100B in the blood?

The answer to this question should be obvious, but it is not. Note that our systematic review shown in Figure 1 identified 44 studies with simultaneous measurement of S100B in the CSF and serum. Many of these studies (one-third of them) report on acute situations of TBI or stroke. These studies associate the serum increase in S100B with the rupture of the BBB, but there is no specific evaluation of this barrier. The other two-thirds of the studies involve brain injury due to a variety of causes, including metabolic, neuroinflammatory (infectious or not), and neurodegenerative disorders. In these investigations, the fragility of the BBB is also frequently mentioned; however, the increase in S100B in the CSF is not always accompanied by an increase in the serum.

In fact, several studies in animal models show that increases in concentrations of S100B in the CSF are not necessarily accompanied by increases in the serum; for example, following LPS administration,[3] in the status epilepticus model with Li-pilocarpine[46] or following intracerebroventricular administration of methylglyoxal.[54,55] The studies with methylglyoxal showed clear disruption of the BBB (as assessed by increased permeability to Evans blue and albumin), without simultaneous leakage of S100B into the serum. Other studies that performed serial analyzes in animal models showed that increases in CSF and serum S100B exhibit different kinetics.[56,57] Studies in patients have shown a delay in changes in serum S100B, which are not compatible with a supposed rupture of the BBB.[50,58–60] Note that many of these studies and conclusions with patients date from prior to the proposal of glymphatic clearance for brain proteins, which currently provides a better interpretation of variations between CSF and serum.[61,62]

Furthermore, in some of the studies where there is probably brain damage, included in Figure 1, increases in serum S100B are observed with no increase in CSF S100B (e.g. Andersson *et al.*[63]), suggesting the contribution of extracerebral sources of S100B. As we saw in section "Serum S100B comes from cerebral and extracerebral sources," adipose tissue is an important source of S100B in serum,[20,64] as S100 proteins play important roles in adipose tissue.[7,18] Secretion of S100B from this tissue may explain increases in serum S100B in cases of trauma (without evidence of brain damage)[65] or mobilization during exercise.[66] However, it is not uncommon for some studies to simply underestimate serum S100B from adipose or other extracerebral sources.[67] It should be emphasized that it is not possible to ignore these sources even when there is an unequivocal increase in CSF S100B resulting from an ischemic stroke or TBI, for example.[68] Adipose tissue occupies a variable amount (from 5% to 30%) of the human body weight, depending on gender. Induction of S100B secretion in the adipose tissue by adrenergic stimulation has been recognized since the 1980s.[69] Sympathetic hyperactivity or systemic adrenergic discharge mediate post-TBI or post-stroke effects on cardiac dysfunction, increased renin, hyperglycemia, and immunological depression. These mechanisms also affect adipose tissue, leading to lipolysis and the release of cytokines and adipokines.[70–72] The central mechanism of sympathetic activation is still poorly understood, but is probably driven by a loss of inhibitory control over excitatory autonomic centers.[73] Figure 3 illustrates how, in these situations of sympathetic hyperactivity, serum S100B levels rise at the expense of central and peripheral secretion. However, although astrocytes may be assumed to contribute more than adipocytes to the serum content of S100B, it is not possible to specify the percentage contribution of each cell type. It should be noted that sympathetic hyperactivation,

by affecting the cardiovascular and renal systems, will affect the blood clearance of the protein, which we will discuss in section "Clearance of S100B in the extracellular medium and from the systemic circulation."

Increases in serum S100B can occur without changes in CSF protein

Some studies have shown increases in serum S100B, independently of increases in the CSF. For example, in rats subjected to fasting for 48 h,[15] in individuals under exercise,[66] or in the case of polytrauma (without brain injury, according to clinical evaluation and neuroimaging)[65] and even in a clinical study of patients undergoing major orthopedic surgeries.[74] In the latter study, serial CSF and serum measurements of S100B were performed, and showed an independent increase in serum protein. These data reinforce the importance of extracerebral sources of S100B.

Conversely, dozens of meta-analyses of neuropsychiatric diseases assume that increases in serum S100B are indicative of damage to the BBB or an increase in CSF. This seems to be true for multiple sclerosis,[9] but in Alzheimer's disease, the increase in serum S100B is not apparently dependent on an increase in CSF S100B[75] (although no study has simultaneously measured S100B protein in the CSF and serum). The significance of increased serum S100B in Alzheimer's disease is still unclear; however, it should be emphasized that although the usefulness of S100B as a peripheral marker may be questionable in Alzheimer's disease, this does not affect the hypothesis that this astroglial protein plays a key role in the pathogenesis of the disease.[76]

The putative transcellular transport of S100B in the brain barriers via RAGE

S100B is transported by endocytosis by a RAGE-dependent mechanism in Schwann cells[77] and astrocytes.[78] This multiligand receptor was previously mentioned in sections "What is the S100B protein?" and "Could the alarmin/cytokine role of S100B per se modulate BBB permeability?," and it is known that S100B and RAGE can mutually regulate their expressions and activations.[6] We therefore postulate that RAGE may participate in the transport of S100B across the BBB, particularly in inflammatory situations where RAGE expression is increased on the endothelium and in astrocytes. However, paracellular transport of S100B across the BBB has not yet been demonstrated.

It is worth mentioning that RAGE ligands, such as β-amyloid peptides, could be transported through the endothelium from outside to inside,[79] as may occur with oxytocin via RAGE.[80] Furthermore, the presence of RAGE has been demonstrated on the epithelium of the choroid plexuses,[81] and an increase in RAGE (and other β-amyloid transporters) at this site has been observed in a murine model of Alzheimer's disease.[82] The presence of this receptor on the BCSFB raises the possibility that S100B-mediated BBB disruption via RAGE (as illustrated in Figure 2) may be accompanied by BCSFB disruption as well; however, this disruption would not involve claudin-5, as this TJ protein is not expressed by the choroid plexus epithelium.

The glymphatic system transports S100B from the brain interstitial fluid to the CSF and then to the bloodstream

Astrocytes closely line the endothelium of cerebral capillaries with extensions (*end feet astrocytes*) and surround the arterioles and venules that cross the nervous parenchyma (*glia limitans*). The space between the glial layer and the vessels, called the Virchow–Robin space (VRS), is a corridor that allows brain interstitial fluid to flow into the CSF. Note that the TJ are in the endothelium, whereas the glial layer has extensive gap junction communication, but lacks TJ. This transport system is called the glymphatic system[83] (see Rasmussen *et al.*[84] for a detailed review). Of note, S100B (and other proteins) does not cross TJ in the VRS corridor. Flow through the VRS goes from the endfoot astrocytes to the glia limitans, driven by a reverse mechanism of astroglial intracellular flow, which is dependent on aquaporin (AQP)-4 water channels and gap junctions (see Figure 4). At the end of the corridor, the S100B enters the subarachnoid space, which is filled with CSF produced in the choroid plexus. Conceptually, the brain interstitial fluid flows into the CSF at this point. The outer layer of cells lining this space, the barrier cells (in the arachnoid mater), possesses the TJs that form the ABB, the outermost brain barrier.

From the subarachnoid space, CSF flows in two ways: (1) to the intradural lymphatics that accompany cranial nerves; and (2) through the arachnoid granulations to the sinuses of the dura mater. In these sites, the structural and functional organization is still little known, but it allows the exit of proteins from the CSF through the trans and paracellular pathways of arachnoid cells. Subsequently, both pathways, flowing into venous vessels, reach the systemic blood circulation. The second pathway develops more after birth. There is no doubt that this pathway is important for amyloid beta clearance, and the accumulation of other proteins, such as S100B, GFAP, and NSE, has been observed in CSF in situations of AQP-4 dysfunction.[85] Interestingly, we observed that AQP-4 blockade with acetazolamide and tetraethylammonium causes an increase in S100B secretion in hippocampal slices.[86] Moreover, intracerebroventricular administration of methylglyoxal, which causes BBB disruption and reduces AQP-4, led to an increase in S100B in the CSF but not in the serum.[54,55]

Just as S100B can play a role (directly and indirectly) in its own transport in the paracellular pathway by modulating TJs (section "Could the alarmin/cytokine role of S100B *per se* modulate BBB permeability?") and, transcellularly, by modulating RAGE expression (section "The putative transcellular transport of S100B in the brain barriers via RAGE"), it can also modulate the expression and activity of AQP-4, which is directly involved in glymphatic drainage. Indeed, the proinflammatory RAGE/NFkB axis in astrocytes, when activated by HMGB1, can lead to AQP-4 expression.[87] This AQP-4 expression axis could be activated by S100B. Furthermore, activation of RAGE, at least in smooth muscle cells, can activate PKA,[88] which phosphorylates AQP-4 at Ser 276 and is involved in the vesicular migration of AQP-4 to the plasma membrane.[89]

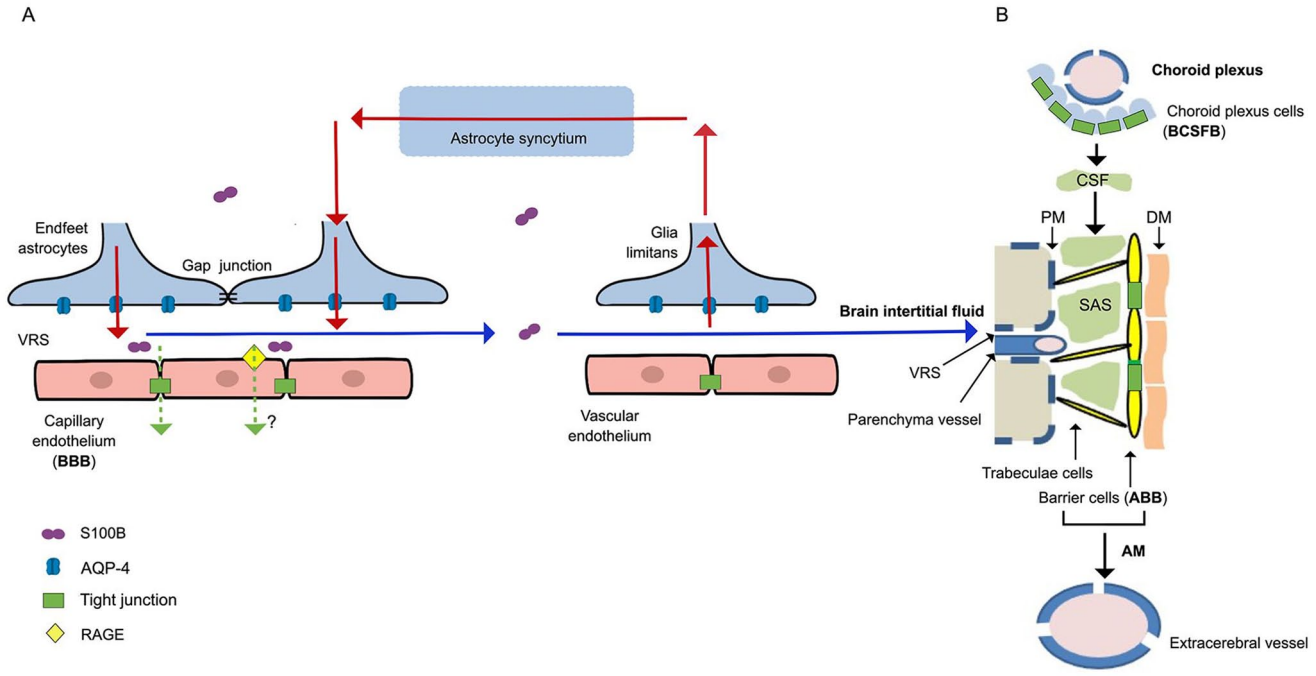

Figure 4. Flow of S100B in the glymphatic system and possibly through the BBB. In panel (a), BBB is represented on the left of the panel, where there is efflux (red arrow) of water via AQP-4 at the astrocytic endfeet. Putatively, in specific situations, S100B could be released directly into the blood at the BBB (via TJ and RAGE, green dashed arrows). The glia limitans are shown on the right, where influx (red arrow) of water occurs through AQP-4. The flow through the astroglial syncytium (red arrow, via AQP-4 and gap junctions) causes movement of interstitial fluid in the RSV (blue arrow), in the opposite direction. In panel (b), the interstitial fluid goes to the subarachnoid space (SAS). The parenchymal vessel can either be an arteriole or a venule. CSF is produced in the choroid plexuses and flows into the SAS. The TJ (green squares) are present in the cells of the choroid plexus (blood cerebrospinal barrier, BCSFB) and arachnoid epithelium (ABB), and in the BBB. From the SAS, brain extracellular proteins reach the systemic circulation, through routes discussed in the text. Notice the fenestrated endothelium in the vessels of the choroid plexuses and extracerebral vessels. Other abbreviations: AM, arachnoid mater; AQP-4, aquaporin-4; DM, dura mater; PM, pia mater; RAGE, receptor for advanced glycated end products; TJ, tight junction.

Clearance of S100B in the extracellular medium and from the systemic circulation

It is currently unknown whether S100B is targeted by any specific extracellular protease. It is likely to be captured by endocytosis and degraded via the endolysosomal pathway. In fact, RAGE undergoes endocytosis and these endosomal vesicles are fused with lysosomes for proteolysis. This has been seen in astrocytes[78] and phagocytes (neutrophils and macrophages).[90]

Calprotectin, a member of the S100 family that is released by neutrophils,[91] has two Cys residues that are susceptible to disulfide bond formation, as with S100B (and other S100 proteins). It was recently shown that formation of calprotectin disulfide (induced in the extracellular environment and amplified in more oxidative inflammatory environments) increases susceptibility to proteolysis.[91] We know that this oxidation at Cys residues of S100B alters the recognition of antibodies used in ELISA;[92] however, whether oxidation increases susceptibility to proteases or the endolysosomal pathway remains to be determined.

It has been demonstrated that serum S100B is eliminated through the kidneys[93,94] and correlates with impaired renal function.[95,96] Since S100 proteins do not bind to major receptors for endocytosis on convoluted proximal tubules, they must be filtered and not reabsorbed. Despite the lack of available data, we should not exclude the possibility of renal protein absorption, since S100B could be internalized by clathrin- and lipid raft-mediated endocytosis.[97] In renal inflammatory processes, especially in diabetic patients and experimental models, there is an increase in RAGE, fibrosis, and AGEs accumulation.[98,99] However, this possibility of absorption in the proximal tubules of S100B needs further investigation.

Conclusions

1. For more than 30 years, increases in S100B in the blood have been associated with acute situations of brain injury, supported by clinical evaluations and neuroimaging. Some studies propose that its use as a brain injury marker may even reduce neuroimaging costs. However, there has always been caution regarding its use, due to the existence of extracerebral sources of S100B and also a "delay" in serum measurements, in relation to probable BBB disruption. This does not preclude the consensus that persistent or late serum elevations ($\geq$ 48 h) generally indicate a worse prognosis for these patients. This mismatch between brain damage, CSF, and serum levels of S100B has been substantially documented and the emergence of the glymphatic system hypothesis, in 2012, has broadened the understanding of the output/drainage of extracellular proteins from the CNS, although even today "BBB rupture" is the major interpretation for increases in serum concentrations of S100B.

2. There are, in the literature, illustrative figures overestimating the direct passage of S100B into the blood, in an inverse analogy to the passage of albumin from the blood into the CSF, during BBB rupture, as evaluated by the CSF/serum ratio. Note that, in this case, the "microfenestration" of the TJ opening in brain capillaries implies that Starling forces act on exchanges, as in peripheral capillaries. The opening of the BBB (within the cerebral parenchyma), however, does not imply that the entire CSF content goes directly into the blood. What could flow into the blood, in this case, may be the local cerebral interstitial fluid. However, even so, this fluid would continue to flow through the VRS corridor into the subarachnoid space. The CSF content of that space goes to the blood. Therefore, even in the case of BBB rupture, there is no way to underestimate the glymphatic system in the transport of S100B and other cerebral extracellular proteins. The rupture of the BBB would allow a localized outflow of S100B into the blood, which may be small in relation to the amount flowing through the glymphatic pathway.
3. In the CNS, S100B is constitutively secreted by astrocytes. Under certain *in vitro* conditions, S100B can be more actively secreted, for example during an ischemic event, AQP-4 dysfunction or $GABA_A$-stimulation with pentylenetetrazol. In addition, some S100B can also be released (not secreted) in situations of death or loss of glial integrity, as with GFAP or NSE in neurons.
4. However, extracellular S100B does not act only as an alarmin, triggering the inflammatory response, it can also activate neuronal and glial survival signaling, whose mechanisms may or may not be dependent on RAGE, its most studied extracellular receptor. Such evidence contradicts the view that S100B is just cell *waste*. The duality of protein action – inflammatory and neurotrophic – can be seen in pharmacological approaches that try to block S100B synthesis or to add exogenous S100B, both aiming for neuroprotection. Furthermore, regardless of being a marker or therapeutic target in acute situations of brain damage, many studies propose a role for this protein in the pathogenesis or monitoring of the treatment of numerous neurological and psychiatric diseases.
5. A limitation of the cell signaling of S100B, discussed so far, is worth mentioning. Activation of RAGE by HMGB1 (a classic alarmin) and S100B has unduly been treated as equivalent. However, these proteins bind to different sites on RAGE, and HMGB1 activates other receptors that S100B does not (e.g. TLR4). Therefore, the activation of RAGE, depending on the activator, and its interaction with other membrane receptors, can result in different cell signaling outcomes.
6. Cerebral S100B reaches the systemic circulation through three possible pathways; via the glymphatic pathway (see Figure 4), by opening the TJ of the brain barriers (paracellular pathway) and transported by RAGE in the endothelium (BBB) and epithelial cells of the choroid plexus (BCSFB) and arachnoidal cells (ABB) (transcellular pathway). Furthermore, S100B *per se* may have direct and indirect modulating effects on the TJ, on the expression of RAGE and on the AQP-4 of the glymphatic system.
7. Extracerebral sources of S100B should not be overlooked, especially adipose tissue. Recent kinetics of S100B in the serum of patients with TBI emphasize that increases from extracerebral sources can overcome increases of cerebral origin. If an algorithm were to be used to assess serum levels of S100B, in addition to brain injury, this would have to take into account age (due to protein ontogeny), weight (due to adipose tissue participation), pigmentation (due to melanocyte activity), renal function, and medications in use that may affect the synthesis and secretion of S100B in the different producing tissues.
8. Finally, in the search for the ideal marker of brain damage, S100B is flawed. However, S100B comes the closest as a biomarker and is the protein that has been studied the most. Like any biomarker, it may be of little use on its own. Experimental and clinical studies have improved our understanding of S100B. We hope this translates into more health benefits in medical practice.

AUTHORS' CONTRIBUTIONS

CAG conceived this mini-review and together with the other authors VGD, AFKV, LR, KMW, LB, MCL, AQS, and AK structured and wrote the text and figures.

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REFERENCES

1. Marenholz I, Heizmann CW, Fritz G. S100 proteins in mouse and man: from evolution to function and pathology (including an update of the nomenclature). *Biochem Biophys Res Commun* 2004;**322**:1111–22
2. Gonçalves CA, Leite MC, Nardin P. Biological and methodological features of the measurement of S100B, a putative marker of brain injury. *Clin Biochem* 2008;**41**:755–63

3. Guerra MC, Tortorelli LS, Galland F, Da Ré C, Negri E, Engelke DS, Rodrigues L, Leite MC, Gonçalves CA. Lipopolysaccharide modulates astrocytic S100B secretion: a study in cerebrospinal fluid and astrocyte cultures from rats. *J Neuroinflammation* 2011;**8**:128
4. Noris-García E, Arce S, Nardin P, Lanigan ME, Acuña V, Gutierrez F, Robinson-Agramonte MA, Gonçalves CA. Peripheral levels of brain-derived neurotrophic factor and S100B in neuropsychiatric systemic lupus erythematous. *Lupus* 2018;**27**:2041–9
5. Wartchow KM, Rodrigues L, Swierzy I, Buchfelder M, de Souza DO, Gonçalves CA, Kleindienst A. Amyloid-β processing in aged S100B transgenic mice is sex dependent. *Int J Mol Sci* 2021;**22**:10823
6. Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, Tubaro C, Giambanco I. S100B's double life: intracellular regulator and extracellular signal. *Biochim Biophys Acta* 2009;**1793**:1008–22
7. Baudier J, Gentil BJ. The S100B protein and partners in adipocyte response to cold stress and adaptive thermogenesis: facts, hypotheses, and perspectives. *Biomolecules* 2020;**10**:843
8. Schroeter ML, Abdul-Khaliq H, Sacher J, Steiner J, Blasig IE, Mueller K. Mood disorders are glial disorders: evidence from in vivo studies. *Cardiovasc Psychiatry Neurol* 2010;**2010**:780645
9. Michetti F, D'Ambrosi N, Toesca A, Puglisi MA, Serrano A, Marchese E, Corvino V, Geloso MC. The S100B story: from biomarker to active factor in neural injury. *J Neurochem* 2019;**148**:168–87
10. Figueira AJ, Saavedra J, Cardoso I, Gomes CM. S100B chaperone multimers suppress the formation of oligomers during Aβ42 aggregation. *Front Neurosci* 2023;**17**:1162741
11. Bancroft EA, De La Mora M, Pandey G, Zarate SM, Srinivasan R. Extracellular S100B inhibits A-type voltage-gated potassium currents and increases L-type voltage-gated calcium channel activity in dopaminergic neurons. *Glia* 2022;**70**:2330–47
12. Balança B, Desmurs L, Grelier J, Perret-Liaudet A, Lukaszewicz AC. DAMPs and RAGE pathophysiology at the acute phase of brain injury: an overview. *Int J Mol Sci* 2021;**22**:2439
13. Vizuete AFK, Fróes F, Seady M, Zanotto C, Bobermin LD, Roginski AC, Wajner M, Quincozes-Santos A, Gonçalves CA. Early effects of LPS-induced neuroinflammation on the rat hippocampal glycolytic pathway. *J Neuroinflammation* 2022;**19**:255
14. Mrak RE, Griffinbc WS. The role of activated astrocytes and of the neurotrophic cytokine S100B in the pathogenesis of Alzheimer's disease. *Neurobiol Aging* 2001;**22**:915–22
15. Netto CB, Conte S, Leite MC, Pires C, Martins TL, Vidal P, Benfato MS, Giugliani R, Gonçalves CA. Serum S100B protein is increased in fasting rats. *Arch Med Res* 2006;**37**:683–6
16. Fujiya A, Nagasaki H, Seino Y, Okawa T, Kato J, Fukami A, Himeno T, Uenishi E, Tsunekawa S, Kamiya H, Nakamura J, Oiso Y, Hamada Y. The role of S100B in the interaction between adipocytes and macrophages. *Obesity* 2014;**22**:371–9
17. Pinto SS, Gottfried C, Mendez A, Gonçalves D, Karl J, Gonçalves CA, Wofchuk S, Rodnight R. Immunocontent and secretion of S100B in astrocyte cultures from different brain regions in relation to morphology. *FEBS Lett* 2000;**486**:203–7
18. Riuzzi F, Chiappalupi S, Arcuri C, Giambanco I, Sorci G, Donato R. S100 proteins in obesity: liaisons dangereuses. *Cell Mol Life Sci* 2020;**77**:129–47
19. Zimmer DB, Song W, Zimmer WE. Isolation of a rat S100 alpha cDNA and distribution of its mRNA in rat tissues. *Brain Res Bull* 1991;**27**:157–62
20. Gonçalves CA, Leite MC, Guerra MC. Adipocytes as an important source of serum S100B and possible roles of this protein in adipose tissue. *Cardiovasc Psychiatry Neurol* 2010;**2010**:790431
21. Capoccia E, Cirillo C, Gigli S, Pesce M, D'Alessandro A, Cuomo R, Sarnelli G, Steardo L, Esposito G. Enteric glia: a new player in inflammatory bowel diseases. *Int J Immunopathol Pharmacol* 2015;**28**:443–51
22. Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* 2006;**7**:41–53
23. Hashimoto Y, Campbell M. Tight junction modulation at the blood-brain barrier: current and future perspectives. *Biochim Biophys Acta Biomembr* 2020;**1862**:183298
24. Greene C, Campbell M. Tight junction modulation of the blood brain barrier: CNS delivery of small molecules. *Tissue Barriers* 2016;**4**:e1138017
25. Günzel D, Yu AS. Claudins and the modulation of tight junction permeability. *Physiol Rev* 2013;**93**:525–69
26. Hashimoto Y, Campbell M, Tachibana K, Okada Y, Kondoh M. Claudin-5: a pharmacological target to modify the permeability of the blood-brain barrier. *Biol Pharm Bull* 2021;**44**:1380–90
27. Rosenthal R, Milatz S, Krug SM, Oelrich B, Schulzke JD, Amasheh S, Günzel D, Fromm M. Claudin-2, a component of the tight junction, forms a paracellular water channel. *J Cell Sci* 2010;**123**:1913–21
28. Nakada T, Kwee IL. Fluid dynamics inside the brain barrier: current concept of interstitial flow, glymphatic flow, and cerebrospinal fluid circulation in the brain. *Neuroscientist* 2019;**25**:155–66
29. Yang C, Hawkins KE, Doré S, Candelario-Jalil E. Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke. *Am J Physiol Physiol* 2019;**316**:C135–53
30. Ponath G, Schettler C, Kaestner F, Voigt B, Wentker D, Arolt V, Rothermundt M. Autocrine S100B effects on astrocytes are mediated via RAGE. *J Neuroimmunol* 2007;**184**:214–22
31. De Souza DF, Wartchow K, Hansen F, Lunardi P, Guerra MC, Nardin P, Gonçalves CA. Interleukin-6-induced S100B secretion is inhibited by haloperidol and risperidone. *Prog Neuropsychopharmacol Biol Psychiatry* 2013;**43**:14–22
32. Yue Q, Hoi MPM. Emerging roles of astrocytes in blood-brain barrier disruption upon amyloid-beta insults in Alzheimer's disease. *Neural Regen Res* 2023;**18**:1890–902
33. Wan W, Chen H, Li Y. The potential mechanisms of Aβ-receptor for advanced glycation end-products interaction disrupting tight junctions of the blood-brain barrier in Alzheimer's disease. *Int J Neurosci* 2014;**124**:75–81
34. Zou Z, Li L, Li Q, Zhao P, Zhang K, Liu C, Cai D, Maegele M, Gu Z, Huang Q. The role of S100B/RAGE-enhanced ADAM17 activation in endothelial glycocalyx shedding after traumatic brain injury. *J Neuroinflammation* 2022;**19**:46
35. Bianchi R, Kastrisianaki E, Giambanco I, Donato R. S100B protein stimulates microglia migration via RAGE-dependent up-regulation of chemokine expression and release. *J Biol Chem* 2011;**286**:7214–26
36. Riuzzi F, Sorci G, Beccafico S, Donato R. S100B engages RAGE or bFGF/FGFR1 in myoblasts depending on its own concentration and myoblast density: implications for muscle regeneration. *PLoS ONE* 2012;**7**:e28700
37. Büyükuysal RL. Protein S100B release from rat brain slices during and after ischemia: comparison with lactate dehydrogenase leakage. *Neurochem Int* 2005;**47**:580–8
38. Gonçalves D, Karl J, Leite M, Rotta L, Salbego C, Rocha E, Wofchuk S, Gonçalves CA. High glutamate decreases S100B secretion stimulated by serum deprivation in astrocytes. *Neuroreport* 2002;**13**:1533–5
39. Tramontina F, Leite MC, Gonçalves D, Tramontina AC, Souza DF, Frizzo JK, Nardin P, Gottfried C, Wofchuk ST, Gonçalves CA. High glutamate decreases S100B secretion by a mechanism dependent on the glutamate transporter. *Neurochem Res* 2006;**31**:815–20
40. Gürsoy M, Büyükuysal RL. Mechanism of S100b release from rat cortical slices determined under basal and stimulated conditions. *Neurochem Res* 2010;**35**:429–36
41. Vizuete AFK, Hansen F, Da Ré C, Leal MB, Galland F, Concli Leite M, Gonçalves CA. GABAA modulation of S100B secretion in acute hippocampal slices and astrocyte cultures. *Neurochem Res* 2019;**44**:301–11
42. Rodrigues L, Wartchow KM, Suardi LZ, Federhen BC, Selistre NG, Gonçalves CA. Streptozotocin causes acute responses on hippocampal S100B and BDNF proteins linked to glucose metabolism alterations. *Neurochem Int* 2019;**128**:85–93
43. Nardin P, Tortorelli L, Quincozes-Santos A, de Almeida LM, Leite MC, Thomazi AP, Gottfried C, Wofchuk ST, Donato R, Gonçalves CA. S100B secretion in acute brain slices: modulation by extracellular levels of Ca(2+) and K (+). *Neurochem Res* 2009;**34**:1603–11
44. Vizuete AFK, Hansen F, Negri E, Leite MC, de Oliveira DL, Gonçalves CA. Effects of dexamethasone on the Li-pilocarpine model of epilepsy: protection against hippocampal inflammation and astrogliosis. *J Neuroinflammation* 2018;**15**:68
45. Kleindienst A, Tolias CM, Corwin FD, Müller C, Marmarou A, Fatouros P, Bullock MR. Assessment of cerebral S100B levels by proton

magnetic resonance spectroscopy after lateral fluid-percussion injury in the rat. *J Neurosurg* 2005;**102**:1115–21
46. Vizuete AFK, Hennemann MM, Gonçalves CA, de Oliveira DL. Phase-dependent astroglial alterations in Li-pilocarpine-induced status epilepticus in young rats. *Neurochem Res* 2017;**42**:2730–42
47. Block F, Dihné M, Loos M. Inflammation in areas of remote changes following focal brain lesion. *Prog Neurobiol* 2005;**75**:342–65
48. Rusnakova V, Honsa P, Dzamba D, Ståhlberg A, Kubista M, Anderova M. Heterogeneity of astrocytes: from development to injury – single cell gene expression. *PLoS ONE* 2013;**8**:e69734
49. Kleindienst A, Hesse F, Bullock MR, Buchfelder M. The neurotrophic protein S100B: value as a marker of brain damage and possible therapeutic implications. *Prog Brain Res* 2007;**161**:317–25
50. Kleindienst A, Meissner S, Eyupoglu IY, Parsch H, Schmidt C, Buchfelder M. Dynamics of S100B release into serum and cerebrospinal fluid following acute brain injury. *Acta Neurochir Suppl* 2010;**106**:247–50
51. Kleindienst A, Grünbeck F, Buslei R, Emtmann I, Buchfelder M. Intraperitoneal treatment with S100B enhances hippocampal neurogenesis in juvenile mice and after experimental brain injury. *Acta Neurochir* 2013;**155**:1351–60
52. Bloomfield SM, McKinney J, Smith L, Brisman J. Reliability of S100B in predicting severity of central nervous system injury. *Neurocrit Care* 2007;**6**:121–38
53. Böhmer AE, Oses JP, Schmidt AP, Perón CS, Krebs CL, Oppitz PP, D'Avila TT, Souza DO, Portela LV, Stefani MA. Neuron-specific enolase, S100B, and glial fibrillary acidic protein levels as outcome predictors in patients with severe traumatic brain injury. *Neurosurgery* 2011;**68**:1624–30; discussion 1630–1
54. Lissner LJ, Rodrigues L, Wartchow KM, Borba E, Bobermin LD, Fontella FU, Hansen F, Quincozes-Santos A, Souza DOG, Gonçalves CA. Short-term alterations in behavior and astroglial function after intracerebroventricular infusion of methylglyoxal in rats. *Neurochem Res* 2021;**46**:183–96
55. Lissner LJ, Wartchow KM, Rodrigues L, Bobermin LD, Borba E, Dias VG, Hansen F, Quincozes-Santos A, Gonçalves CA. Acute methylglyoxal-induced damage in blood-brain barrier and hippocampal tissue. *Neurotox Res* 2022;**40**:1337–47
56. Kleindienst A, Ross Bullock M. A critical analysis of the role of the neurotrophic protein S100B in acute brain injury. *J Neurotrauma* 2006;**23**:1185–200
57. Tanaka Y, Marumo T, Omura T, Yoshida S. Relationship between cerebrospinal and peripheral S100B levels after focal cerebral ischemia in rats. *Neurosci Lett* 2008;**436**:40–3
58. Kleindienst A, Schmidt C, Parsch H, Emtmann I, Xu Y, Buchfelder M. The passage of S100B from brain to blood is not specifically related to the blood-brain barrier integrity. *Cardiovasc Psychiatry Neurol* 2010;**2010**:801295
59. Brandner S, Thaler C, Buchfelder M, Kleindienst A. Brain-derived protein concentrations in the cerebrospinal fluid: contribution of trauma resulting from ventricular drain insertion. *J Neurotrauma* 2013;**30**:1205–10
60. Steinacker P, Weidehaas K, Cepek L, Feneberg E, Kretzschmar HA, Otto M. Influence of the blood-CSF-barrier function on S100B in neurodegenerative diseases. *Acta Neurol Scand* 2013;**128**:249–56
61. Lindblad C, Nelson DW, Zeiler FA, Ercole A, Ghatan PH, von Horn H, Risling M, Svensson M, Agoston DV, Bellander BM, Thelin EP. Influence of blood-brain barrier integrity on brain protein biomarker clearance in severe traumatic brain injury: a longitudinal prospective study. *J Neurotrauma* 2020;**37**:1381–91
62. Thelin EP, Nelson DW, Bellander BM. A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. *Acta Neurochir* 2017;**159**:209–25
63. Andersson M, Oras J, Thörn SE, Karlsson O, Kälebo P, Zetterberg H, Blennow K, Bergman L. Signs of neuroaxonal injury in preeclampsia – a case control study. *PLoS ONE* 2021;**16**:e0246786
64. Steiner J, Schiltz K, Walter M, Wunderlich MT, Keilhoff G, Brisch R, Bielau H, Bernstein HG, Bogerts B, Schroeter ML, Westphal S. S100B serum levels are closely correlated with body mass index: an important caveat in neuropsychiatric research. *Psychoneuroendocrinology* 2010;**35**:321–4
65. Ohrt-Nissen S, Friis-Hansen L, Dahl B, Stensballe J, Romner B, Rasmussen LS. How does extracerebral trauma affect the clinical value of S100B measurements? *Emerg Med J* 2011;**28**:941–4
66. Koh SX, Lee JK. S100B as a marker for brain damage and blood-brain barrier disruption following exercise. *Sports Med* 2014;**44**:369–85
67. Pham N, Fazio V, Cucullo L, Teng Q, Biberthaler P, Bazarian JJ, Janigro D. Extracranial sources of S100B do not affect serum levels. *PLoS ONE* 2010;**5**:e12691
68. Undén J, Bellner J, Reinstrup P, Romner B. Serial S100B levels before, during and after cerebral herniation. *Br J Neurosurg* 2004;**18**:277–80
69. Suzuki F, Kato K, Nakajima T. Hormonal regulation of adipose S-100 protein release. *J Neurochem* 1984;**43**:1336–41
70. Wang YY, Lin SY, Chuang YH, Chen CJ, Tung KC, Sheu WH. Adipose proinflammatory cytokine expression through sympathetic system is associated with hyperglycemia and insulin resistance in a rat ischemic stroke model. *Am J Physiol Endocrinol Metab* 2011;**300**:E155–63
71. Wang H, Deng QW, Peng AN, Xing FL, Zuo L, Li S, Gu ZT, Yan FL. β-arrestin2 functions as a key regulator in the sympathetic-triggered immunodepression after stroke. *J Neuroinflammation* 2018;**15**:102
72. Louraoui SM, Fliyou F, Aasfara J, El Azhari A. Paroxysmal sympathetic hyperactivity after traumatic brain injury: what is important to know? *Cureus* 2022;**14**:e24693
73. Meyfroidt G, Baguley IJ, Menon DK. Paroxysmal sympathetic hyperactivity: the storm after acute brain injury. *Lancet Neurol* 2017;**16**:721–9
74. Danielson M, Wiklund A, Granath F, Blennow K, Mkrtchian S, Nellgård B, Oras J, Jonsson Fagerlund M, Granström A, Schening A, Rasmussen LS, Erlandsson Harris H, Zetterberg H, Ricksten SE, Eriksson LI. Neuroinflammatory markers associate with cognitive decline after major surgery: findings of an explorative study. *Ann Neurol* 2020;**87**:370–82
75. Bellaver B, Ferrari-Souza JP, Uglione da Ros L, Carter SF, Rodriguez-Vieitez E, Nordberg A, Pellerin L, Rosa-Neto P, Leffa DT, Zimmer ER. Astrocyte biomarkers in Alzheimer disease: a systematic review and meta-analysis. *Neurology* 2021;**2021**:12109
76. Wilcock DM, Griffin WST. Down's syndrome, neuroinflammation, and Alzheimer neuropathogenesis. *J Neuroinflammation* 2013;**10**:84
77. Perrone L, Peluso G, Melone MA. RAGE recycles at the plasma membrane in S100B secretory vesicles and promotes Schwann cells morphological changes. *J Cell Physiol* 2008;**217**:60–71
78. Lasič E, Galland F, Vardjan N, Šribar J, Križaj I, Leite MC, Zorec R, Stenovec M. Time-dependent uptake and trafficking of vesicles capturing extracellular S100B in cultured rat astrocytes. *J Neurochem* 2016;**139**:309–23
79. Zhang YL, Wang J, Zhang ZN, Su Q, Guo JH. The relationship between amyloid-beta and brain capillary endothelial cells in Alzheimer's disease. *Neural Regen Res* 2022;**17**:2355–63
80. Yamamoto Y, Higashida H. RAGE regulates oxytocin transport into the brain. *Commun Biol* 2020;**3**:70
81. Pascale CL, Miller MC, Chiu C, Boylan M, Caralopoulos IN, Gonzalez L, Johanson CE, Silverberg GD. Amyloid-beta transporter expression at the blood-CSF barrier is age-dependent. *Fluids Barriers CNS* 2011;**8**:21
82. González-Marrero I, Giménez-Llort L, Johanson CE, Carmona-Calero EM, Castañeyra-Ruiz L, Brito-Armas JM, Castañeyra-Perdomo A, Castro-Fuentes R. Choroid plexus dysfunction impairs beta-amyloid clearance in a triple transgenic mouse model of Alzheimer's disease. *Front Cell Neurosci* 2015;**9**:17
83. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci Transl Med* 2012;**4**:147ra111
84. Rasmussen MK, Mestre H, Nedergaard M. Fluid transport in the brain. *Physiol Rev* 2022;**102**:1025–151
85. Ferrara M, Bertozzi G, Volonnino G, Di Fazio N, Frati P, Cipolloni L, La Russa R, Fineschi V. Glymphatic system a window on TBI pathophysiology: a systematic review. *Int J Mol Sci* 2022;**23**:9138

86. Zanotto C, Abib RT, Batassini C, Tortorelli LS, Biasibetti R, Rodrigues L, Nardin P, Hansen F, Gottfried C, Leite MC, Gonçalves CA. Non-specific inhibitors of aquaporin-4 stimulate S100B secretion in acute hippocampal slices of rats. *Brain Res* 2013;**1491**:14–22
87. Tang Z, Yang G, Liao Z, Chen F, Chen S, Wang W, Huo G, Sun X, Wang X. Tanshinone IIA reduces AQP4 expression and astrocyte swelling after OGD/R by inhibiting the HMGB1/RAGE/NF-κB/IL-6 pro-inflammatory axis. *Sci Rep* 2022;**12**:14110
88. Yu T, Wang Y, Qian D, Sun X, Tang Y, Shen X, Lin L. Advanced glycation end products impair Ca2+ mobilization and sensitization in colonic smooth muscle cells via the CAMP/PKA pathway. *Cell Physiol Biochem* 2017;**43**:1571–87
89. Kitchen P, Salman MM, Halsey AM, Clarke-Bland C, MacDonald JA, Ishida H, Vogel HJ, Almutiri S, Logan A, Kreida S, Al-Jubair T, Winkel Missel J, Gourdon P, Törnroth-Horsefield S, Conner MT, Ahmed Z, Conner AC, Bill RM. Targeting aquaporin-4 subcellular localization to treat central nervous system edema. *Cell* 2020;**181**:784–99.e19
90. Andersson U, Yang H, Harris H. High-mobility group box 1 protein (HMGB1) operates as an alarmin outside as well as inside cells. *Semin Immunol* 2018;**38**:40–8
91. Hoskin TS, Crowther JM, Cheung J, Epton MJ, Sly PD, Elder PA, Dobson RCJ, Kettle AJ, Dickerhof N. Oxidative cross-linking of calprotectin occurs in vivo, altering its structure and susceptibility to proteolysis. *Redox Biol* 2019;**24**:101202
92. Leite MC, Galland F, Brolese G, Guerra MC, Bortolotto JW, Freitas R, Almeida LMV, de Gottfried C, Gonçalves CA. A simple, sensitive and widely applicable ELISA for S100B: methodological features of the measurement of this glial protein. *J Neurosci Methods* 2008;**169**:93–9
93. Jönsson H, Johnsson P, Höglund P, Alling C, Blomquist S. Elimination of S100B and renal function after cardiac surgery. *J Cardiothorac Vasc Anesth* 2000;**14**:698–701
94. Ytrebø LM, Nedredal GI, Korvald C, Holm Nielsen OJ, Ingebrigtsen T, Romner B, Aarbakke J, Revhaug A. Renal elimination of protein S-100beta in pigs with acute encephalopathy. *Scand J Clin Lab Invest* 2001;**61**:217–25
95. Gross S, Homan van der Heide JJ, van Son WJ, Gans RO, Foell D, Navis G, Bakker SJ. Body mass index and creatinine clearance are associated with steady-state serum concentrations of the cell damage marker S100B in renal transplant recipients. *Med Sci Monit* 2010;**16**:CR318–24
96. Li JP, Lu L, Wang LJ, Zhang FR, Shen WF. Increased serum levels of S100B are related to the severity of cardiac dysfunction, renal insufficiency and major cardiac events in patients with chronic heart failure. *Clin Biochem* 2011;**44**:984–8
97. Zhang Y, Zhu J, Xu H, Yi Q, Yan L, Ye L, Zhang X, Xie M, Tan B. Time-dependent internalization of S100B by mesenchymal stem cells via the pathways of clathrin- and lipid raft-mediated endocytosis. *Front Cell Dev Biol* 2021;**9**:674995
98. Liu J, Huang K, Cai GY, Chen XM, Yang JR, Lin LR, Yang J, Huo BG, Zhan J, He YN. Receptor for advanced glycation end-products promotes premature senescence of proximal tubular epithelial cells via activation of endoplasmic reticulum stress-dependent p21 signaling. *Cell Signal* 2014;**26**:110–21
99. Haraguchi R, Kohara Y, Matsubayashi K, Kitazawa R, Kitazawa S. New insights into the pathogenesis of diabetic nephropathy: proximal renal tubules are primary target of oxidative stress in diabetic kidney. *ACTA Histochem Cytochem* 2020;**53**:21–31