Multimodality Monitoring, Inflammation, and Neuroregeneration in Subarachnoid Hemorrhage

BACKGROUND: Stroke, including subarachnoid hemorrhage (SAH), is one of the leading causes of morbidity and mortality worldwide. The mortality rate of poor-grade SAH ranges from 34% to 52%. In an attempt to improve SAH outcomes, clinical research on multimodality monitoring has been performed, as has basic science research on inflammation and neuroregeneration (which can occur due to injury-induced neurogenesis). Nevertheless, the current literature does not focus on the integrated study of these fields. Multimodality monitoring corresponds to physiological data obtained during clinical management by both noninvasive and invasive methods. Regarding inflammation and neuroregeneration, evidence suggests that, in all types of stroke, a proinflammatory phase and an anti-inflammatory phase occur consecutively; these phases affect neurogenesis, which is also influenced by other pathophysiological features of stroke, such as ischemia, seizures, and spreading depression.

OBJECTIVE: To assess whether injury-induced neurogenesis is a prognostic factor in poor-grade SAH that can be monitored and modulated.

METHODS: We propose a protocol for multimodality monitoring-guided hypothermia in poor-grade SAH in which cellular and molecular markers of inflammation and neuroregeneration can be monitored in parallel with clinical and multimodal data.

EXPECTED OUTCOMES: This study may reveal correlations between markers of inflammation and neurogenesis in blood and cerebrospinal fluid, based on clinical and multimodality monitoring parameters.

DISCUSSION: This protocol has the potential to lead to new therapies for acute, diffuse, and severe brain diseases.

KEY WORDS: Neural stem cells, Neurocritical care, Neurogenesis, Neuroinflammation, Neuroprotection

The protocol proposed by this study is entitled, “Multimodality monitoring, inflammation and neuroregeneration in subarachnoid hemorrhage.” The identification number is RBR55t5v9; Registro Brasileiro de Ensaios Clinicos (ReBEC, Brazilian Clinical Trials Registry); December 20, 2012. Dr Teixeira was the study coordinator, and research was conducted at the Division of Neurosurgery Clinics, Hospital das Clínicas, Faculty of Medicine, University of São Paulo, São Paulo, Brazil and at the Department of Surgery, Santa Casa Faculty of Medical Sciences, São Paulo, Brazil.

GENERAL INFORMATION

Protocol Title

Multimodality monitoring, inflammation and neuroregeneration in subarachnoid hemorrhage.

Identifying Number

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Funding Agency

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazilian National Council for Scientific and Technological

ABBREVIATIONS: BBB, blood-brain barrier; CPP, cerebral perfusion pressure; EEG, electroencephalography; ICP, intracranial pressure; IL, interleukin; MCA, middle cerebral artery; SAH, subarachnoid hemorrhage; SD, spreading depression; SGZ, subgranular zone; SVZ, subventricular zone; TCD, transcranial Doppler
NEUROGENESIS IN SUBARACHNOID HEMORRHAGE

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RATIONALE AND BACKGROUND INFORMATION

Epidemiology of Subarachnoid Hemorrhage
Stroke is a leading cause of morbidity and mortality worldwide. Subarachnoid hemorrhage (SAH), which accounts for approximately 5% of all types of stroke, generally results from the spontaneous hemorrhage of a brain aneurysm. The annual incidence of SAH is 10/100,000 worldwide. Patients with poor-grade SAH, in-hospital mortality rates range from 34% (Hunt and Hess grade IV, stupor) to 52% (Hunt and Hess grade V, coma); however, 3 decades ago, these rates were approximately twice as high.2

Pathophysiology of SAH
Cerebral Blood Flow and Metabolism
Cerebral hemodynamics are linked to brain metabolism. Regional blood flow is proportional to the demand for oxygen and glucose, which is the principal energy substrate for neurons. Histologically, the link between cerebral hemodynamics and brain metabolism is explained by the neurovascular unit, which constitutes the blood-brain barrier (BBB). The vascular components of the neurovascular unit are the endothelial cells, contractile cells that surround the endothelial cells (myocytes in the arterioles and pericytes in the capillaries), and the basal lamina of the vessel. The neural components of the neurovascular unit are the astrocytes, which project throughout the vessels, and the neurons, which are more peripherally located and are in contact with the astrocytes. Between the vascular and neural components lies the Virchow-Robin space, which is an inflection of the leptomeninges over the pial vessels at the point where these vessels enter the brain parenchyma. Therefore, in theory, lesions of the neurovascular unit can release molecules and cells that are detectable by laboratory tests into both the cerebrospinal fluid (CSF) and the bloodstream.

Overall, cerebral blood flow (CBF) remains relatively stable at between 80% and 120% of baseline, despite variations in the cerebral perfusion pressure (CPP). This mechanism is known as cerebral autoregulation or, more specifically, static cerebral autoregulation, and was first described by Lassen in 1959. The CPP is calculated by subtracting the intracranial pressure (ICP) from the mean arterial pressure. Static cerebral autoregulation is maintained when CPP values are within the approximate range of 50 to 150 mm Hg. Outside of this range, the CBF varies as a function of the CPP. Nevertheless, in recent years, a phenomenon known as dynamic cerebral autoregulation, in which rapid and subtle changes in the CBF occur, has been described. Such short-term CBF variations occur within a CPP range where the long-term CBF remains stable; in other words, dynamic and static cerebral auto-regulation mechanisms are simultaneous.

Clinically, dynamic cerebral autoregulation can be evaluated by transcranial Doppler (TCD), using parameters such as the pulsatility index and the CO2 reactivity, which are based on the principle of an inverse relationship between blood vessel flow and resistance. The pulsatility index is given by the difference between the systolic and diastolic velocities in a given artery (generally the middle cerebral artery [MCA]), divided by the mean velocity. In addition, CO2 reactivity can evaluate the cerebrovascular reserve capacity; when CO2 reactivity is preserved, variations in arterial CO2 tension elicit a proportional blood velocity response in blood vessels such as the MCA.

Under pathological conditions, such as SAH, the oxygen supply may not be sufficient to meet the energetic demand in certain regions of the brain. Prolonged hypoxia leads to a gradual change in neurons, as originally described by Opitz and Schneider in 1950. At a flow of approximately 55 mL/100 g per min, neurons are functionally inactive but remain viable, forming the ischemic penumbra. Below 35 mL/100 g per min, anaerobic glycolysis predominates. Below 15 mL/100 g per min, the cell membrane is damaged, which leads to cell death. Nevertheless, adequate blood flow and an adequate supply of glucose and oxygen do not necessarily translate into adequate energy production, as demonstrated in recent studies. This phenomenon, known as the metabolic penumbra, has been attributed to mitochondrial dysfunction.

In summary, an imbalance between the energy substrate’s supply and demand appears to be the principal trigger for brain cell injury. When this imbalance occurs, protective mechanisms are activated. However, the persistence of such an imbalance leads to the progression of cell injury mechanisms, including the cytotoxic effects of glutamate, massive calcium influx into the cell, free radical formation, proinflammatory cytokine production, apoptosis, and necrosis. Although such factors are interrelated, they are only pieces of a puzzle that is far from complete. In broad terms, such factors are common to the pathophysiology of all types of severe acute brain injury, including SAH.

Phases of SAH
Classically, in addition to hydrocephalus and rebleeding, the principal neurological complication of SAH is vasospasm. However, 2 changes that precede the onset of vasospasm, ie, early brain injury and cortical spreading depression (depolarization), have recently been described as relevant factors for SAH-related morbidity.
Early brain injury refers to pathophysiological mechanisms that occur within the first 72 hours after a stroke. In patients with SAH, aneurysmal bleeding initially leads to ischemia, BBB damage, and cytotoxic edema. A cascade of molecular and cellular events may occur in this phase. Among such events, apoptosis, triggered principally by the activation of the cytochrome c and caspase pathways, plays a significant role.

Spreading depression (SD) was first described in 1944 by the Brazilian physiologist Leão. In models of ischemia, SD is a mechanism of injury in which waves of depolarization are generated from a central region of necrosis to the periphery of the ischemic penumbra. The pathophysiological substrate of SD is an exacerbated glutamate release. In a study of SAH patients, SD was detected by using chronic electrocorticography in the period following aneurysm clipping. In this study, a strong correlation between the frequency of SD and the incidence of delayed ischemic neurological deficits was observed.

Vasospasm may occur from 3 to 5 days after SAH; its incidence is higher between posthemorrhage days 5 and 14, progressively lower in the following 2 weeks, and practically absent in later periods. Vasospasm has been attributed to hemorrhagic degradation products. The iron, heme, and biliverdin that are released during degradation contribute to the formation of free radicals. More recently, other mechanisms for vasospasm have been described, with endothelin appearing to play a role in increasing cerebral vascular tone; in addition, inflammatory and apoptotic activities mediate cell proliferation and the increased number of myocytes in the arteriolar walls.

SAH also causes systemic changes. Clinically, there is an association between SAH and the systemic inflammatory response syndrome. Moreover, SAH patients develop myocardial dysfunction, which manifests as electrocardiographic changes and altered cardiac output. Right atrial distension may increase the production of brain natriuretic peptide, which is produced almost exclusively by the heart in humans. The high incidence of cerebral salt-wasting syndrome in SAH is partially explained by the interrelationship of these phenomena.

Inflammation

During the first week after acute cerebral ischemia, the central nervous system is affected by a proinflammatory phase. Inflammatory activity is increased by various factors, namely: (1) production of reactive oxygen species; (2) production of cytokines and chemokines by neurons and astrocytes; (3) activation of the endogenous macrophages of brain parenchyma (microglia); (4) rupture of the BBB; and (5) endothelial cell expression of selectins, especially intercellular adhesion molecule-1. The proinflammatory phase is characterized by the participation of enzymes (matrix metalloproteinases 2 and 9), cytokines (tumor necrosis factor alpha, interferon gamma, interleukin [IL]-1β, IL-2, and IL-6), chemokines (monocyte chemoattractant protein-1 and stromal cell-derived factor-1 alpha), and cells (microglia and certain lymphocytes).

The proinflammatory phase is followed by an anti-inflammatory phase, which is similar to sepsis. In cerebral ischemic injury, the activity of IL-10 and regulatory T lymphocytes predominates. In fact, higher levels of IL-10 and greater numbers of regulatory T lymphocytes in the ischemic brain lead to a lower stroke volume. However, from a systemic standpoint, the anti-inflammatory phase contributes to the onset of infection.

Contradictory effects of certain cytokines have been reported. IL-6 has been reported to both improve and worsen cerebral ischemic injury. The effects of tumor necrosis factor alpha seem to depend on the type of receptor that is activated. Microglia and chemokines have different effects that appear to be phase dependent. In the proinflammatory phase, microglia and chemokines attract leukocytes and prevent newly formed neurons (neuroblasts) from surviving. However, in the anti-inflammatory phase, they contribute to the survival, migration, maturation, and integration of migrating neuroblasts.

Neuroregeneration

Adult neurogenesis in humans has been recently described. Adult mammalian neurogenesis occurs physiologically in the subventricular zone (SVZ) and in the subgranular zone (SGZ) of the dentate gyrus in the hippocampus, but it may also occur in pathological situations. Thus, neurogenesis is increased in models of cerebral ischemia. In humans, ischemic stroke triggers the emergence of cells around the site of the injury. These cells are positive for neurogenesis-related markers, such as βIII-tubulin, but the true significance of this finding is controversial because βIII-tubulin is also present in normal human brains. Specifically, in SAH, brain samples from SAH patients have been reported to contain an increased number of neural stem cells.

The pathophysiological mechanisms that are present in SAH can trigger an increase in neurogenesis. SD has been reported to trigger increased neurogenesis in the SVZ and in the SGZ of the dentate gyrus. In addition, the subpial zone, which is supposedly nonneurogenic under physiological conditions, has been reported to show the same type of response to SD. One controversy in this line of research has regarded the location of neurogenic niches in normal and diseased human brains.

To clarify this issue, as shown in Figure 1, our group mapped neurogenic niches in humans. We have confirmed the existence of neurogenesis markers in the SVZ and SGZ. Likewise, nestin, a neural stem cell marker, has been detected in the isocortex (which is supposedly nonneurogenic area under physiological conditions) of a sample obtained from a patient with epilepsy (data not shown). Analogously, epileptic seizures in patients with SAH may trigger the same type of response.

The correlation between individual variations in the injury-induced neurogenesis and functional outcome is unknown. Our study suggests that this correlation may be significant, because we have shown that the human brain expresses neurogenesis markers in a continuous area between the hippocampus and hypothalamus. This area is larger than previously thought and involves the circumventricular organs, where the BBB is absent. The mechanisms underlying possible neurogenesis arising in the
circumventricular organs may be enhanced during injury-induced neurogenesis by the breakdown of the BBB. Under pathological conditions, the relationship between hematopoiesis, neurogenesis, and vasculogenesis occurs at different levels. Thus, sublethal ischemia induces collateral vessel formation in the pial vessels (ischemic preconditioning). Granulocyte colony-stimulating factor, a growth factor with a previously studied neurogenic potential, is involved in this process. Additionally, erythropoietin (which was discovered in studies of hematopoiesis) was tested in a phase II clinical trial of patients with SAH who had improved outcomes. It has been suggested that erythropoietin may improve outcomes through a neuroprotective effect. In contrast, vascular endothelial growth factor, which stimulates vasculogenesis, seems to contribute to worse SAH outcomes.

In summary, SAH triggers an intense inflammatory response, initiating a proinflammatory phase that is followed by an anti-inflammatory phase. In each phase, various cells, cytokines, and chemokines predominate. Some of these cells and molecules are present in both phases, and their effects depend on the context. Initially, they are proinflammatory. Subsequently, they are thought to participate in mechanisms of neuroregeneration, although this has yet to be confirmed, especially in humans.

**Multimodality Monitoring**

Current State of Multimodality Monitoring in Neurocritical Care

The most important aspect of the management of poor-grade SAH is clinical evaluation. However, clinical evaluation is not sufficient for understanding the true condition of the central nervous system or anticipating declines in function. Therefore,
monitoring of physiological parameters is required. In practice, because of the risks and benefits of the individual methods, a combination of methods is used. Obtaining data in this manner is known as multimodality monitoring. In the literature, multimodality monitoring is considered one of the principal strategies for defining both the treatment and outcome in some severe neurological diseases.

**Multimodality Monitoring Methods**

**Transcranial Doppler.** TCD is an ultrasound-based method for evaluating cerebral hemodynamics. As previously mentioned, TCD provides data on the CBF, cerebral autoregulation, and cerebral hemodynamic reserve. In SAH cases, TCD is widely used to detect vasospasm, based on parameters such as the Lindegaard ratio (the ratio between the MCA flow velocity and the internal carotid artery flow velocity). TCD is particularly recommended for the diagnosis of severe vasospasm.

**Continuous Electroencephalography.** Continuous electroencephalography (EEG) is a noninvasive method. In poor-grade SAH, this method is principally indicated for detecting epileptic seizures, predicting the onset of delayed cerebral ischemia, and

### TABLE 1. Intensive Care Unit Notes

| Clinical and Laboratorial Parameters | Assessment |
|-------------------------------------|------------|
| 1. General: length of stay; days after hemorrhage; aneurysm location; vasospasm* (yes or no); other diagnoses | Once a day |
| 2. Severity scores: APACHE II; SOFA; SAPS 3 | Once a day |
| 3. Lines, tube, and drains: type/days after insertion | Once a day |
| 4. Neurological: sedation (drug/dosage); SAS; RSS; GCS; pupils; motor deficit | Every 1 h |
| 5. Cardiovascular: SBP; DBP; MAP; HR; rhythm; CVP; Cl; ΔSV | Continuous |
| Fluid balance | Every 1 h |
| ECG: troponin; BNP; chest x-ray (congestion: yes or no) | Once a day |
| Drugs: antiarrhythmics; DVT prophylaxis; vasoactive drugs | Once a day |
| 6. Respiratory: MV (yes or no); MV mode; FiO2; PEEP; respiratory rate; ΔP (above PEEP); ETCO2; oxygen saturation | Continuous |
| Arterial blood gas analysis | Every 12 h |
| 7. Renal: urinary output | Every 1 h |
| Na+ (in mEq/L): | Once a day |
| Na+ = 140-150 and ΔNa+ in 24 h < 5 | Every 12 h |
| Na+ = 135-139 or 151-155 or ΔNa+ in 24 h = 5.1-7.5 | Every 8 h |
| Na+ = 130-134 or 155-160 or ΔNa+ in 24 h = 7.6-10.0 | Every 6 h |
| Na- < 130 or >160 or ΔNa- in 24 h > 10 | Every 4 h |
| 8. Gastrointestinal tract: diet; bowel habit; ulcer prophylaxis | Once a day |
| Albumin | Every 2 days |
| Hepatic enzymes | Every 4 days |
| 9. Infectious: rectal temperature | Continuous |
| White blood cell count; CRP; Svo2; arterial lactate; BE; antibiotics; focus of infection; infectious agent; temperature control measures | Once a day |
| 10. Hematological: hemoglobin; hematocrit; platelets; PT; APTT | Once a day |
| 11. Endocrine: capillary blood glucose* | 2/2 h |
| TSH; free thyroxine (T4) | Every 2 days |
| Cortisol (collection between 8:00 AM and 8:30 AM) | Every 7 days |
| 12. Locomotor: edema/signs of DVT | Once a day |
| 13. Dermatological: scars (location/severity)/allergic reactions | Once a day |
| 14. Other | Continuous |

*APACHE, acute physiology and chronic health evaluation; APTT, activated partial thromboplastin time; BE, base excess; BNP, brain natriuretic peptide; Cl, cardiac index; CRP, C-reactive protein; CVP, central venous pressure; DBP, diastolic blood pressure; DVP, deep vein thrombosis; ECG, electrocardiogram; ETCO2, end-tidal carbon dioxide tension; FiO2, fraction of inspired oxygen; GCS, Glasgow Coma Scale; HR, heart rate; Ca, ionized calcium; MAP, mean arterial pressure; MV, mechanical ventilation; Na+, urinary sodium; PEEP, positive end-expiratory pressure; PT, prothrombin time; RSS, Ramsay Sedation Scale; SAPS, simplified acute physiology score; SAS, Sedation-Agitation Scale; SBP, systolic blood pressure; SOFA, sequential organ failure assessment; Svo2, mixed venous oxygen saturation; TSH, thyroid-stimulating hormone; ΔP, variation in pressure; ΔSV, variation in systolic volume.

*Minimal frequency of assessment; the frequency of assessment of any physical examination item is determined by the intensive care unit team.

*Clinical vasospasm (focal deficit or reduced level of consciousness) or delayed cerebral ischemia (including ischemia detected only by cranial CT). If hyperosmolar therapy is not indicated, maintain Na+ in the 140 to 150 mEq/L range; due to progressive evidence of an association between high levels of magnesium and outcome improvement, maintain Mg2+ in the 2.4 to 4.8 mg/dL range (in cases of decreased blood pressure or arrhythmia, maintain normal levels). If >180 mg/dL, consider a continuous intravenous regular insulin protocol.
Continuous EEG can predict delayed cerebral ischemia as early as 2 days before its onset. The principal change, observed in over 50% of the patients, is a decrease in the alpha/delta ratio. The methods for quantifying such patterns would alert the intensivist, precluding the need for interpretation by an EEG specialist, which is a disadvantage of EEG. Similarly, EEG data may be quantified by the bispectral index, which is used to determine the level of sedation, although its prolonged use in the intensive care unit setting has yet to be validated. Another parameter for the evaluation of sedation is the presence of burst suppression. EEG data acquisition may be hampered by local factors, such as scalp edema and space limitations due to the presence of catheters and surgical incisions.

Monitoring of ICP, Brain Temperature, and Brain Tissue Oxygenation ($P_{ti}O_2$). The ICP, brain temperature, and $P_{ti}O_2$ are monitored by intracranial catheters. The most widely used of these 3 methods is ICP monitoring. ICP values >20 mm Hg are associated with a poor outcome. An intraventricular catheter is considered to be the most accurate type of ICP-monitoring catheters and has the advantage of allowing CSF drainage to manage intracranial hypertension. A disadvantage of the ventricular catheter is an increased likelihood of infection, especially after 5 to 7 days of use.

Brain temperature monitoring is warranted because of its association with outcomes in poor-grade SAH. Brain temperature monitoring is a less established and more invasive method of predicting outcome compared with body temperature monitoring. Nevertheless, the exact relationship between the brain and body temperature measurements in severe neurological conditions is unknown, and their values may not match. Thus, in poor-grade SAH cases where ICP monitoring is performed, brain temperature monitoring does not pose additional or unnecessary risks, provided that the same catheter is used to monitor both the ICP and the brain temperature. Finally, $P_{ti}O_2$ can add to multimodality monitoring by displaying tissue hypoxia, eg, when its value in the white matter is below 20 mm Hg. A limitation of monitoring the brain temperature and $P_{ti}O_2$ is the lack of validated parameters. Moreover, the $P_{ti}O_2$ catheter cannot detect hypoxia that occurs in areas distant from its position in the brain parenchyma, which is another limitation because brain tissue oxygenation may be heterogeneous in SAH.

**Brain Microdialysis.** Microdialysis was introduced to neuroscience in the 1970s. It was first used clinically in the 1990s. Microdialysis is performed by placing a 1-mm probe into the area of interest. In patients with SAH, the probe can be inserted during craniotomy. Another option is to use a screw to attach the microdialysis probe to the remaining catheters for multimodality monitoring ($P_{ti}O_2$, brain temperature, and parenchymal ICP). The consensus is that the probe must be placed in the area that is fed by the artery with the aneurysm. The risks of infection and bleeding are low. Some authors have reported that microdialysis probes can be maintained for up to approximately 10 days.

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### TABLE 2. Admission, Aneurysm Occlusion, and Catheter Placement

| Phases                                 | Procedures |
|----------------------------------------|------------|
| First phase: clinical stabilization     | Otrachael intubation (ETCO$_2$ = 35 mm Hg) |
|                                        | MAP $\geq$ 90 mm Hg, SBP $\leq$ 160 mm Hg (if necessary, 5-20 mg bolus of labetolol every 15 min; maintenance: 2 mg/min, maximum of 300 mg/day) |
|                                        | Sedation up to RSS score 5 |
|                                        | Obtain large-bore venous access |
|                                        | Placement of indwelling urinary catheter and enteral tube |
|                                        | Include in the prescription: |
|                                        | Maintenance fluid: 1000 mL of SS + 40 mL of 20% NaCl + 10 mL of 19.1% KCl + 20 mL of 10% MgSO$_4$ administered i.v. 1.5 mL/kg/h |
|                                        | To avoid hypovolemia, administer SS every 6 h, as necessary |
|                                        | Administer $\geq$ 500 mL/day of SS for every degree above 37°C |
|                                        | 60 mg of nimodipine through enteral tube every 4 h |
|                                        | 40 mg of simvastatin through enteral tube once a day |
|                                        | 15 mg/kg of i.v. phenytoin in SS for 30 min + 100 mg administered i.v. every 8 h |
| Second phase: aneurysm occlusion and catheter placement | CT angiography or brain angiography |
| Craniotomy (consider embolization)     | Trepanation for placement of ICP (preferentially EVD), $P_{ti}O_2$ (white matter) and microdialysis probes (grey matter) |

$^1$CT, computed tomography; ETCO$_2$, end-tidal arterial carbon dioxide tension; EVD, external ventricular drain; ICP, intracranial pressure; MAP, mean arterial pressure; MF, maintenance fluid; $P_{ti}O_2$, brain tissue oxygenation; RSS, Ramsay Sedation Scale; SBP, systolic blood pressure; SS, saline solution.

$^2$Place in an area perfused by the artery containing the aneurysm.

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monitoring sedation. Epileptic seizures are common and worsen outcomes, regardless of the frequent absence of clinical repercussions. Claassen et al. conducted a study of 108 patients with SAH and diagnosed clinical epileptic seizures in 19% of these patients. Of the entire sample, 18% had nonconvulsive seizures and 13% had nonconvulsive status epilepticus. Periodic epileptiform discharges, principally periodic lateralized epileptiform discharges, occurred in 23% of the patients. Such discharges are supposed to correspond to a spectrum between interictal and ictal activity.
Microdialysis probes allow for the collection of potentially cytotoxic neurotransmitters (glutamate), energy substrates (glucose), and glucose metabolites (lactate and pyruvate), as well as molecular markers of membrane damage (glycerol). Monitoring glucose, lactate, and pyruvate levels allows for the evaluation of glycolysis. In the absence of oxygen or when the mitochondrial oxygen uptake is inadequate, pyruvate is converted into lactate, a process that is far less energy efficient. In practice, the principal parameter evaluated in the diagnosis of metabolic stress is the lactate/pyruvate ratio, and values higher than 40 are classified as altered. In a study of patients with SAH, the metabolic parameters studied through microdialysis allowed the authors to predict vasospasm an average of 7.8 hours before its onset. Despite its potential, brain microdialysis requires further validation. Moreover, another limitation of this method is that it provides data on only a single area of the brain parenchyma.

Aspects of the Treatment of Poor-Grade SAH

Evidence-Based Medicine

The American Stroke Association has recently systematized the guidelines for SAH. The Brain Trauma Foundation has outlined practices for monitoring ICP and cerebral hemodynamics, although such practices are not directly related to poor-grade SAH. For more controversial issues, such as multimodality monitoring, guidelines based on currently available evidence can be found. However, high-level evidence and grades of recommendation remain rare in SAH, trauma, and multimodality monitoring guidelines. This partially explains why a recent study on numerous centers worldwide found discrepancies in the approaches to the most important aspects of SAH.

Neuroprotection

The approach to treating poor-grade SAH involves measures that are aimed at avoiding the progression of secondary ischemic injury. Such measures include sedation, hyperosmolar therapy, and monitoring clinical parameters. Moreover, the neuroprotective role of hypothermia has become clear. Hypothermia has been reported to act on virtually all secondary injury mechanisms, including the modulation of the inflammatory response. The principal adverse effects of hypothermia include cardiac arrhythmia, coagulopathies, and a higher risk of infection. In addition, effective temperature control can be complex in clinical practice. We speculate that some disadvantages of hypothermia may be overcome by the development of a straightforward method for cooling neural tissue (eg, CSF cooling), which avoids the technical obstacle of the low thermal conductivity of the skull.

### TABLE 3. Practices Guided by Multimodality Monitoring

| Methods             | Initial Parameters                                      | Interventions                                                                 |
|---------------------|--------------------------------------------------------|-------------------------------------------------------------------------------|
| Cranial CT          | Various possible imaging changes                       | Neurosurgical evaluation                                                    |
| TCD                 | LR > 6, PI > 1.2, or CO2 reactivity < 3%              | Algorithms for CVSP and neuroprotection                                      |
| cEEG                | Epileptic seizures/status epileptic                    | Phenytoin (± another AED, if necessary)                                      |
|                     |                                                       | Midazolam (maximum, 0.6 mg/kg per h)                                         |
|                     |                                                       | Pentobarbital                                                                |
| ICP                 | >50% decrease in the ADR                              | Algorithms for CVSP and neuroprotection                                      |
|                     |                                                       | Open EVD, algorithm for neuroprotection, adjust CPP and CBV (ETCO₂, PEEP)    |
| CPP                 | <70 mm Hg                                             | Control ICP or MAP (volume or VADs)                                          |
|                     | >80 mm Hg                                             | Avoid hypervolemia and VADs                                                   |
| P<sub>1</sub>O₂      | <20 mm Hg                                             | Check/adjust SaO₂ and P<sub>1</sub>O₂                                      |
| Microdialysis       | Lactate/pyruvate ratio >40 (consider the remaining parameters of microdialysis) | Algorithms for CVSP and neuroprotection                                      |
|                     |                                                       | Check capillary blood glucose levels                                         |
| Brain temperature   | ≥37.5°C                                                | Perform the interventions for P<sub>1</sub>O₂                                |
|                     |                                                       | Up to 4 g/day of dipyrone                                                    |
|                     |                                                       | Up to 3.2 g/day of ibuprofen                                                |
|                     |                                                       | Surface cooling method + control of chills                                   |

ADR, alpha/delta ratio; AED, antiepileptic drug; cEEG, continuous electroencephalography; CBV, cerebral blood volume; CPP, cerebral perfusion pressure; CT, computed tomography; CVSP, clinical vasospasm prophylaxis; ETCO₂, end-tidal carbon dioxide tension; ICP, intracranial pressure; LR, Lindegaard ratio; MAP, mean arterial pressure; Pa<sub>O₂</sub>, arterial oxygen tension; PEEP, positive end-expiratory pressure; PI, pulsatility index; P<sub>1</sub>O₂, brain tissue oxygenation; SaO₂, arterial oxygen saturation; TCD, transcranial Doppler; VAD, vasoactive drugs.

1Percentage of increase in velocity for every 1-mm Hg increase in arterial carbon dioxide tension.

3Percentage of increase in velocity for every 1-mm Hg increase in arterial carbon dioxide tension.
Correlations between functional outcomes and data obtained from both multimodality and laboratory monitoring may help to overcome a major obstacle in the development of new stroke therapies, namely, the need for a large number of patients to confirm the effect of any given therapeutic approach. Thus, the number of patients required to confirm whether a given treatment improves clinical vasospasm is estimated to be more than 5000. Although controversial, an alternative approach is the use of a surrogate biomarker. The physiological, cellular, and molecular analyses proposed in the protocol below constitute potential biomarkers for SAH management.

**STUDY GOALS AND OBJECTIVES**

This project has 1 clinical and 1 experimental goal. The clinical goal is to assess the safety and potential improvement in poor-grade SAH outcomes via a protocol of multimodal monitoring-guided therapeutic hypothermia. The experimental goal is to clarify the mechanisms of inflammation and neuroregeneration involved in poor-grade SAH, emphasizing the potential development of novel therapies for acute and severe neurological injuries.

The specific objectives of this study are:

- Evaluate the complications and technical difficulties of the proposed protocol;
- Compare clinical monitoring methods in terms of their predictive value for vasospasm and relevance for management;
- Analyze the effects of rigorous control of brain temperature, \( P_{\text{ETCO}_2} \) and metabolic stress on the clinical outcomes and inflammatory response;
- Correlate pro- and anti-inflammatory markers with neurogenesis-related markers in blood and cerebrospinal fluid; and
- Correlate inflammation and neurogenesis-related markers with delayed brain ischemia, multimodality monitoring data, and clinical outcomes.
STUDY DESIGN

Protocol Proposal

General Aspects

The number of studies investigating each of the items addressed in this study is considerable. However, few studies have attempted to simultaneously investigate these items. Our objective was to do so and to reach conclusions that may have potential practical applications. Therefore, we deemed it more appropriate to present the proposal of a protocol for the multimodality monitoring of patients with poor-grade SAH and a concomitant analysis of the molecular and cellular markers related to inflammation and neuroregeneration. As with any other protocol, the decision to implement the particular protocol proposed here should be made on a case-by-case basis, taking into account multiple and unpredictable clinical variables. In addition, the present protocol is intended as a starting point and is open to criticism and adaptations. Our protocol may be adapted to other acute, diffuse, and severe neurological diseases. Nevertheless, certain relevant guidelines should be followed, namely the following: (1) it should be implemented only at centers with extensive experience in SAH; (2) a multidisciplinary team should be available; (3) it should be approved by the local research ethics committee; and (4) suitable financial support should be sought.

Type of Clinical Trial and Inclusion and Exclusion Criteria

Due to the clinical characteristics of the patients, the present protocol is suitable for a prospective, observational study that has an end point by October 2016. The expected sample size is 20 patients. The inclusion criteria are as follows: (1) patients who are 18 to 65 years of age; (2) hospitalization within the first 72 hours after the first hemorrhage; (3) presentation with Hunt and Hess grade IV or V; and (4) presentation with an anterior circulation aneurysm. The exclusion criteria are: (1) a neurosurgical procedure at another facility due to SAH (eg, placement of an external ventricular drain); (2) presentation with a severe systemic disease; and (3) presentation with either a Glasgow coma scale score of 3 or pathological posturing accompanied by bilateral mydriasis with fixed pupils.

METHODOLOGY

Clinical Data

We will collect data on the clinical evolution (Table 1), laboratory tests (eg, those specified in Table 1), prescriptions, and interventions guided by multimodality monitoring. Continuous measurements will be stored by commercially available monitoring systems that simultaneously display vital signs, mechanical ventilation data, and the majority of neurological...
multimodality monitoring parameters. Likewise, an EEG device will separately be used to continuously store data. TCD and a cranial CT scan will be performed at least daily and every 3 days, respectively.

The treatment of poor-grade SAH, guided by multimodality monitoring, is presented as follows: admission, aneurysm occlusion, and catheter placement (Table 2); parameter definition and management regarding multimodality monitoring methods (Table 3); and algorithms for clinical vasospasm prophylaxis (Figure 2), neuroprotection (Figure 3), and removal of multimodality monitoring catheters (Figure 4).

### Inflammation and Neuroregeneration Markers

As seen in Table 4, the inflammation and neuroregeneration markers are of a molecular and cellular nature. The molecular markers will be determined by immunoenzymatic assays, whereas the cellular markers will be detected by flow cytometry. A total of 4 mL of CSF will be collected on the day that the ventricular catheter is inserted and on the day that it is removed, as well as once in between these 2 days. On the same days, 4 mL of blood will be collected. Another 4 mL of blood will be collected on post-SAH days 14, 21, 28, 90, and 180. The material (CSF or blood, 1.5 mL per sample) will be stored at −80°C for immunoenzymatic assays.

After collecting samples from groups of 3 patients, immunoenzymatic assays will be performed for all molecules. The remaining samples will be stored at 4°C for weekly flow cytometry. Controls for the cells studied in the blood will be performed by using samples from volunteers. For molecules present in the CSF, 15 samples from patients with an indication for lumbar puncture (eg, screening for SAH, meningitis, or polyradiculoneuritis), whose test results are normal, will be evaluated; this figure has been estimated on the basis of blood sample controls, as described in the directions for use of commercially available reagents.

### DISCUSSION

Various conditions treated in the scope of neurocritical care have high morbidity and mortality rates. One of the greatest challenges is poor-grade SAH. The challenge lies in the development of methods for monitoring and treating SAH as well as in our understanding of the pathophysiology of SAH. Therefore, SAH is a potential model for the standardization of multimodality monitoring as well as for studies of the influence of inflammation and neuroregeneration mechanisms in stroke.

### Trial Status

At the moment, we have concluded the basic science study that underpins this clinical trial. The current phase of this project involves human resources and infrastructure aspects.
Safety Considerations

Treatment will be discussed daily between the staff of the critical care unit and the researchers who participate in the study, who will also be available in loco or by phone for additional clinical evaluation, if necessary. Families will receive daily reports on the clinical status of their relative who is enrolled in the study, and a contact number will be provided for further questions.

Follow-Up

Research participants will be followed in the hospital where they receive treatment via regular consultations at the neurosurgery outpatient units or via evaluation at the casualty department, if necessary.

Data Management and Statistical Analysis

Data will be stored in 2 personal computers located in the institutions that participate in the study and will be accessible by the authors of this article or staff members who are formally authorized by one of the authors (M.J.T.).

The end point of this project is mortality within 180 days after SAH. Mortality, functional outcomes, and the presence of delayed ischemic neurological deficits will be correlated with the alterations in multimodal monitoring parameters and inflammatory and neuroregeneration markers.

Quality Assurance

The study follows the guidelines of Good Clinical Practices. The study coordinator will supervise data management, and files will be synchronized with a backup system. Adverse events will be communicated to the Agência Nacional de Vigilância Sanitária (Anvisa, National Agency of Sanitary Vigilance) and to the Research and Ethical Committees of both hospitals, who are also responsible for periodic analysis and revision of the study progress.

Expected Study Outcome

The expected study outcome is the identification of a correlation between mortality and functional outcomes and neuroregeneration markers. This finding would reveal potential biomarkers for monitoring poor-grade SAH cases and would raise the possibility that injury-induced neurogenesis plays a role in clinical recovery after severe brain injury.

Duration of the Project

This project will take up to 36 months. The first 12 months were dedicated to performing the basic science research that underpins the clinical trial, setting up the infrastructure, and importing equipment and reagents. The remaining 24 months will be spent in the proper development of the clinical trial.

Project Management

The project involves the collaboration of a multidisciplinary team under the supervision of Dr Teixeira.

Ethics

This protocol has been approved by the Brazilian Clinical Trials Registry (Rebec), a primary registry that participates in the WHO International Clinical Trials Registry Platform.

Disclosures

This work was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazilian National Council for Scientific and Technological Development; grant 401002/2013-6). The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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**Acknowledgments**

The authors thank Adriana Satie Morimoto, MD; Alison Colquhoun, PhD; Carmen Lisa Jorge, MD, PhD; Eberwal Gadelphia Figueiredo, MD, PhD; Edson Bor Seng Shu, MD, PhD; Juan Antonio Castro Flores, MD; Marzoc de Queiroz Teles Gomes, MD; Luiz Henrique Martins Castro, MD, PhD; Mari Cleide Sogayar, PhD; Sheila Aparecida Siqueira, MD, PhD; and Vinicius Monteiro de Paula Guirado, MD.