Cerebrovascular Reactivity Measured by Near-Infrared Spectroscopy

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Background and Purpose—The pressure reactivity index (PRx) describes cerebral vessel reactivity by correlation of slow waves of intracranial pressure (ICP) and arterial blood pressure. In theory, slow changes in the relative total hemoglobin (rTHb) measured by near-infrared spectroscopy are caused by the same blood volume changes that cause slow waves of ICP. Our objective was to develop a new index of vascular reactivity, the hemoglobin volume index (HVx), which is a low-frequency correlation of arterial blood pressure and rTHb measured with near-infrared spectroscopy.

Methods—Gradual hypotension was induced in piglets while cortical laser-Doppler flux was monitored. ICP was monitored, and rTHb was measured continuously using reflectance near-infrared spectroscopy. The HVx was recorded as a moving linear correlation between slow waves (20 to 300 seconds) of arterial blood pressure and rTHb. Autoregulation curves were constructed by averaging values of the PRx or HVx in 5-mm Hg bins of cerebral perfusion pressure.

Results—The laser-Doppler flux-determined lower limit of autoregulation was 29.4±6.7 mm Hg (±SD). Coherence between rTHb and ICP was high at low frequencies. HVx was linearly correlated with PRx. The PRx and HVx both showed higher values below the lower limit of autoregulation and lower values above the lower limit of autoregulation. Areas under the receiver operator characteristic curves were 0.88 and 0.85 for the PRx and HVx, respectively.

Conclusions—Coherence between the rTHb and ICP waveforms at the frequency of slow waves suggests that slow waves of ICP are related to blood volume changes. The HVx has potential for further development as a noninvasive alternative to the PRx. (Stroke. 2009;40:1820-1826.)

Key Words: autoregulation ■ cerebral blood flow ■ hemoglobin ■ hypotension ■ near-infrared spectroscopy ■ neonate ■ piglet

Cerebrovascular autoregulation is the preservation of nearly constant cerebral blood flow (CBF) across a range of cerebral perfusion pressure (CPP). Autoregulation is a vital protective function of the cerebral vasculature, and when this function fails, the brain is at risk of injury. Autoregulation is mediated by vascular reactivity, which is defined as low-frequency diameter changes in resistance vessels in response to changes in arterial blood pressure (ABP). Various measures of dynamic autoregulation have been developed and reviewed. Assessments of CBF autoregulation are distinct from assessments of vascular reactivity. Autoregulation metrics gauge the transmission of low-frequency CPP changes to CBF, whereas a measure of vascular reactivity quantifies the effect of low-frequency ABP changes on cerebral arteriolar activity. Therefore, in most physiological instances, limits of pressure reactivity are wider than limits of autoregulation.

Intact autoregulation can be demonstrated by absent correlation between slow waves of CPP and a surrogate of CBF. Conversely, impaired autoregulation—which can be referred to as a state of pressure passivity—results in positive correlation between slow waves of CPP and CBF. In contrast, in assessing vascular reactivity, the effect of changes in ABP on a surrogate measure of cerebral blood volume (CBV) is measured. These low-frequency changes in CBV are attributed to the volume changes caused by the dynamic collective vascular radius. When the vasculature is pressure passive, low-frequency CBV waves are coherent and in phase with the ABP. However, when the vasculature is pressure-reactive, blood volume in the brain is reactive to the ABP. Perfect pressure reactivity theoretically results in a blood volume waveform that is still coherent with the ABP at low frequencies but is 180° phase-shifted. With linear correlation, therefore, pressure passivity yields a correlation of 1, whereas perfect pressure reactivity yields a correlation of −1 between the CBV and ABP.
The prototypical monitor of vascular reactivity is the pressure reactivity index (PRx).\(^5\) Intracranial pressure (ICP) is used as the surrogate of CBV to derive the PRx, which is a moving linear correlation between slow waves of ABP and ICP. A positive PRx, indicating pressure passivity, is associated with death in adults with traumatic brain injury. A negative PRx, indicating pressure reactivity, is associated with survival in adults with traumatic brain injury.\(^7\) The PRx can delineate a range of perfusion pressure with maximal vascular reactivity in most patients with traumatic brain injury, and deviation from this optimal perfusion pressure is associated with death and persistent vegetative state.\(^8\) The strength of these data led to a citation in the guidelines for the management of severe traumatic brain injury from The Brain Trauma Foundation with a new option for autoregulation monitoring to fine-tune CPP goals.\(^9\)

Clinical application of the PRx is limited by the requirement for invasive ICP monitoring. Many patients at risk of devastating atraumatic neurological injuries do not or cannot have ICP monitors. Examples include patients on cardiopulmonary bypass and premature infants at risk for developing intraventricular hemorrhage and periventricular leukomalacia. It is reasonable to hypothesize that these patients would benefit from clarification of optimal blood pressure goals by a nonintracranially invasive form of monitoring vascular reactivity. We have previously reported the use of noninvasive cerebral oximetry as a surrogate of CBF in a monitor of autoregulation—the cerebral oximetry index.\(^10\) Our objective with the present work is to use near-infrared reflectance spectroscopy (NIRS) to trend changes in CBV (relative total hemoglobin in the reflectance arc) to create a near-infrared-based monitor of vascular reactivity—the hemoglobin volume index (HVx).

We hypothesized that slow waves of relative total hemoglobin (rTHb) measured with NIRS would be coherent with slow waves of ICP. Predicated on demonstrating this low-frequency coherence, we further hypothesized that the HVx, a moving linear correlation between slow waves of rTHb and ABP, would have a strong correlation to the PRx, thereby providing a relatively noninvasive method by which to quantify vascular reactivity in patients without ICP monitors. Finally, we hypothesized that the HVx, like the PRx, would accurately detect the lower limit of cerebrovascular autoregulation in a swine model of induced hypotension.\(^11\) We tested these hypotheses by continuously measuring rTHb with NIRS while synchronously measuring ICP and ABP in piglets as ABP was lowered below the lower limit of autoregulation (LLA).

**Materials and Methods**

All experiments were approved by the Johns Hopkins University Animal Care and Use Committee. All procedures conformed to the standards of animal experimentation from the National Institutes of Health.

**Anesthesia**

Methods of anesthesia and surgical preparation have been previously described and published.\(^10\) Eight piglets, 5 to 10 days old and weighing 2.34±0.47 kg (mean±SD), were anesthetized with inhaled 5% isoflurane, 50% nitrous oxide, and 50% oxygen. Tracheostomy was performed and mechanical ventilation initiated and adjusted to maintain arterial pH between 7.35 and 7.45 and PaO\(_2\) between 200 and 300 mm Hg. Maintenance anesthesia consisted of 0.8% isoflurane, 50% nitrous oxide, 50% oxygen, fentanyl (25-μg bolus followed by 25-μg/h infusion), and vecuronium (5-mg bolus followed by 2-mg/h infusion). The fentanyl infusion was adjusted to remain between 10 and 50 μg/h to keep the heart rate less than 200 beats per minute and to maintain normal blood pressure. When the blood pressure was actively lowered by inflating a balloon catheter in the inferior vena cava, tachycardia was permitted as an expected response to the induced reduction in preload. The primarily narcotic-based anesthetic technique, supplemented with a relatively low concentration of isoflurane, ensured the animals’ comfort while minimizing the cerebrovascular response to the volatile anesthetic agent. Piglets were placed on a warming pad to keep their brain and rectal temperatures at 38.5° to 39.5°C.

**Surgery**

A femoral central venous catheter for drug infusion and a femoral arterial catheter for blood pressure monitoring and blood sampling were placed. A 5-Fr esophageal balloon catheter (Cooper Surgical, Trundall, Conn) was placed in the contralateral groin and threaded into the inferior vena cava. Slow, controlled systemic hypotension was induced by slowly inflating the balloon catheter in the inferior vena cava. A craniotomy was performed 4 mm rostral and 4 mm lateral to the bregma at midline for placement of an external ventricular drain catheter to monitor ICP. A second craniotomy was performed 4 mm lateral to the ventricular drain for placement of a laser-Doppler flux (LDF) probe (Moor Instruments, Devon, UK). The dura mater was incised, and the LDF probe was advanced to contact the surface of the frontoparietal cortex and secured in place with rubber washers cemented to the cranium. A third small craniotomy in the occipital cranium just lateral to midline was performed for a brain temperature probe. All craniotomy sites were sealed with dental cement to preserve the integrity of the intracranial compartment. The skin was reapplied to the skull and sutured closed for heat retention.

Neonatal optodes for the FORE-SIGHT near-infrared spectroscopic monitor (CASMED, Inc, Branford, Conn) were placed contralateral to the craniotomy sites, lying across the frontal and parietal lobes, sutured in place, and shielded with opaque black nylon until there was light. The differential optical distance of the neonatal sensor was 25 mm. Optimal probe placement was then verified with a CO\(_2\) challenge as previously described.\(^10\)

**Signal Sampling**

ABP, ICP, and LDF measurements were sampled from an analog-to-digital converter at 100 Hz using ICM+ software (Cambridge University, Cambridge, UK, www.neurosurg.cam.ac.uk/icmplus). CPP was calculated as (ABP−ICP) and recorded every 10 seconds. Cerebral oximetry and rTHb values were synchronously sampled from the digital output of the NIRS monitor with a refresh rate of 30/min.

**rTHb Measurement**

We used the FORE-SIGHT monitor to record rTHb using an algorithm developed at the University College of London and used in the Hamamatsu NIRO-500/1000 series NIRS systems.\(^12-14\) We used a multivariate form of this algorithm with 3 wavelengths (780, 805, 850 nm) to solve 2 unknowns (\(\Delta[Hb]\) and \(\Delta[HbO_2]\)), which when added yielded rTHb.

An alternative method of determining rTHb was much simpler. We used transmittance from a nearly isobestic wavelength (805 nm) of infrared light as an approximation of rTHb. The index of autoregulation derived from this approximation was indistinguishable from that obtained with the more complicated University College of London rTHb algorithm. Although neither of these measurements of rTHb concentration are calibrated and an artificial nonlinearity is introduced by using simple transmittance (instead of its logarithm in the modified Beer-Lambert equation) to trend blood.
volume, both measures of rTHb had the same accuracy when used to
describe vascular reactivity. Because the rTHb change during auto-
regulation is a small fraction of the absolute hemoglobin, a logarith-
mic transformation may not be necessary. Moreover, the phase of the
blood volume waveform is more important than the power when
describing vascular reactivity. Results in this article are reported
using the University College of London algorithm, but it should be
noted that receiver operating characteristics (ROC) of the derived
indices using the University College of London method versus
transmittance of 805 nm light for rTHb were identical (data not
shown).

Determining the LLA
The balloon catheter in the inferior vena cava was inflated with
saline through a syringe pump infusion to slowly lower ABP. The
goal was to decrease ABP over approximately 3 hours to achieve a
quasisteady-state CPP and capture an adequate sample of spontane-
ous slow wave fluctuations (Figure 1A). The recording period during
which ABP was lowered was 3 hours 39 minutes±48 minutes
(mean±SD). A scatterplot of 1-minute averaged LDF versus CPP
was generated for each piglet. The CPP at the intersection of the 2
regression lines with the lowest combined residual squared error was
defined as the LLA (Figure 1B). The slope of the line describing the
range of intact autoregulation was not required to be zero.

Low-Pass Filtering and Calculation of the PRx
and HVx
The signals were time-integrated and resampled as nonoverlapping
10-second mean values to eliminate high-frequency waves and,
specifically, harmonics from pulse and respiration. Oscillatory
changes that occur <0.05 Hz are still detected with this low-pass
filtering method.

The vascular reactivity indices were calculated as follows. A
continuous moving Pearson correlation was performed between slow
waves (20 to 300 seconds) of ICP and ABP to calculate PRx and
between slow waves of rTHb and ABP to calculate HVx. Consecu-
tive paired 10-second averaged values from 300-second epochs
generated 30 data points for inclusion in each Pearson coefficient
used to determine the PRx and HVx. As explained at the beginning
of this article, positive values of PRx or HVx indicate impaired
vascular reactivity, and negative values indicate intact vascular
reactivity.

Figure 1. Determining the lower limit of autoregulation. A, ABP (mm Hg), ICP (mm Hg), rTHb (AU), and LDF (AU) were measured con-
tinuously in a newborn piglet as ABP was gradually lowered to zero. Prominent spontaneous slow waves with a period between 1 and 5
minutes are seen. Slow waves in the ABP are out of phase with the ICP and rTHb until the ABP is critically low at the end of the exper-
iment, when ABP, ICP, and rTHb slow waves are all in phase. B, LDF measurements from A were plotted as a function of CPP for each
animal individually. The intersection of the 2 best-fit lines with the lowest combined residual error squared is the LLA for the animal. In
this subject, the LLA was at a CPP of 34 mm Hg (dashed vertical line), and below this line, LDF was passive to CPP.
Comparison of rTHb and ICP Waveforms
The waveforms of ICP and rTHb were collected from each animal during a 60-minute period of rest without manipulation of ABP. Coherence between the 2 waveforms was assessed using the Welsh method with 40 overlapping segments (at 10% overlap) with a spectral range from 0.004 to 0.05 Hz having total hemoglobin as the input and ICP as the output (Figure 2).

Correlation of the PRx and HVx
All paired values of the PRx and HVx from the entire duration of the 8 experiments were included in a linear (Spearman) correlation and Bland-Altman analysis using Prism software (GraphPad, San Diego, Calif).

Receiver Operating Characteristics
PRx and HVx were calculated every 10 seconds from overlapping 300-second analysis periods and sorted into 5-mm Hg bins according to the CPP at which they were recorded. For each piglet, average values for PRx and HVx were reported at each CPP bin. After calculating the LLA from the LDF data as described, the PRx and HVx were divided into data sets above and below the LLA. Prism software was used to generate the ROC of the PRx and HVx from this dichotomized data set, in which values obtained below the LLA are labeled measurements in the lost autoregulatory state and values obtained above the LLA are labeled measurements in the intact autoregulatory state.

Results
Arterial pH, blood gas tensions, hemoglobin concentration, and brain temperatures were similar among all piglets (Table). The piglets had an average ICP of 7.8±2.8 mm Hg (±SD). On average, the LLA occurred at a CPP of 29.4±6.7 mm Hg (±SD) for the 8 animals studied, a result that is consistent with our prior study of piglets.10 Spontaneous low-frequency (0.004 to 0.05 Hz) changes in rTHb were usually in phase with ICP (Figure 2). Harmonics in this spectral range had periodicity ranging from 20 seconds to 250 seconds, the period of slow waves. Coherence scores between rTHb and ICP were divided into frequency bins and reported using box whiskerplots. The result of this cross-spectral analysis, averaging the data from all 8 animals, is shown in Figure 3. Slow waves of ICP in these piglets occurred with longer periods (often ≥50 seconds) than those seen in humans with ICP monitoring. This period corresponds to a frequency of <0.02 Hz, and it can be seen from our data that the coherence scores between the ICP and rTHb are higher in this range. This demonstration is a sine qua non for our study design: slow waves of ICP are the signal used for the PRx, and finding coherence between ICP and rTHb slow waves raises the possibility that rTHb can be used in place of ICP to quantify vascular reactivity.

The relationship of HVx with PRx was examined using all 2242 measurements from the 8 experiments (Figure 4). Plotting these paired measurements of HVx and PRx yielded a robust correlation with a Spearman rank r value of 0.73. Increases in PRx are sensitive for detecting the LLA in piglets.11 To determine if HVx is equally sensitive, results

| Table. Physiological Parameters of the Piglets at Normal and Low CPP |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| CPP >40 mm Hg   | pH              | P_{a}CO_{2}, mm Hg | P_{a}O_{2}, mm Hg | Hemoglobin, g/dL |
| 7.35±0.08       | 38±3            | 279±47           | 8.5±1.4          |
| CPP <40 mm Hg   | 7.35±0.08       | 41±4             | 267±32           | 7.9±1.5         |
| Brain Temperature, °C | 38.5±1.0       |                  |                  | 38.3±1.1        |
ROC curves were determined from the data after binning for each animal. The vascular reactivity index value (PRx or HVx) at the LLA for the ROC analysis (Figure 5). In clinical practice, the PRx is commonly sorted in this way to determine “CPP_opt,” which is defined as the CPP bin with the most negative average PRx flanked by progressively positive values of PRx. Reporting the data and ROC analysis from these histograms is, therefore, an analysis of the accuracy of the method as it has been deployed. Areas under the ROC curves were 0.88 (95% CI, 0.82 to 0.96) and 0.85 (95% CI, 0.75 to 0.94) for the PRx and HVx, respectively. Converting these ROC curves into statements of sensitivity and specificity requires the assignment of a cutoff value for the PRx and HVx. Such thresholds have not been rigorously established, but mortality after traumatic brain injury has been shown to increase from 20% to 70% when comparing patients with a PRx below and above 0.3. With the PRx, sensitivity and specificity when using 0.3 as the cutoff between intact and impaired vascular reactivity in the ROC curves shown in Figure 6 are 80% and 79%, respectively. The same cutoff is 77% sensitive and 84% specific with the HVx. Higher thresholds increase specificity, and in our study, a cutoff of 0.4 increases specificity to 90% for the PRx and 94% for the HVx.

**Discussion**

The results of the experiments presented here have 2 important implications. First, the results imply that the nonintracranially invasive HVx has a potential clinical role in the care of patients at risk of neurological injury. Second, the conceptual model used to derive the PRx is now better supported by the close agreement between the HVx and PRx.

Given the amount and quality of patient data linking a positive PRx to poor outcome, it is perhaps academic to validate the conceptual model that was used to create the PRx 10 years ago. However, the lack of a continuous and convenient metric for CBV, other than the ICP itself, has precluded such a validation. The PRx is, in theory, a measure of the responses of cerebral resistance arterioles to slow waves of ABP that are commonly seen in monitored patients. The assumption inherent in this model is that slow waves of ICP, specifically waves with a period of between 20 and 300 seconds, are the result of CBV changes, which are in turn caused by changes in the collective arteriolar diameter. Our finding of coherence between rTHb and ICP at the slow-wave frequency in these animals links these slow ICP waves to slow blood volume waves. The finding of a reactive relationship between blood volume and ABP above the LLA and a passive relationship between blood volume and ABP below the LLA at the frequency of slow waves specifically implicates resistance arterioles as the vascular compartment responsible for the slow waves seen in both the blood volume and ICP. Taken together, these results defend the assumptions made when using the PRx.

We have shown, in the naïve animal, that the HVx is an excellent nonintracranially invasive alternative to the PRx. One difference between the PRx and HVx that should be considered is the regional specificity of the HVx, which only describes vasculature in the reflective path of infrared light between the optodes of the NIRS-based monitor. The PRx uses a global measurement of ICP, potentially influenced by the collective activity of all cerebral vessels. The implications of this difference have not been determined and are probably situationally specific. For instance, after trauma, is the HVx obtained over injured versus noninjured brain tissue different? If so, which of these regional indices approximates the global PRx and which can be used to guide therapy to the best outcome? These questions are important for future studies of this modality with bilateral NIRS monitoring.
success has previously been reported in quantifying relative CBV noninvasively with ultrasound time-of-flight measurement. This measurement was then used to measure vascular reactivity as a phase shift between the relative blood volume and the ABP changes at the respiratory frequency as opposed to analyzing at the frequency of slow waves as is done with the PRx and the HVx. The theoretical advantage of using respiratory frequency waveform analysis is the regular periodicity when compared with the sporadic nature of slow waves. However, the phase shift between CBV and ABP resulting from vascular reactivity is “incomplete” at this frequency (that is to say that the high-pass filter used to describe the effect of autoregulation on ABP–CBV transmission has its transition band around this frequency). Furthermore, the pediatric range of respiratory frequency is higher and more variable than the adult range. Therefore, the phase shift of blood volume to blood pressure variation at the pediatric respiratory frequency is likely smaller and more variable than that reported in adults. Until the effect of changing respiratory frequency on the range of normal phase shift between respiration and CBV is delineated, these spectra cannot be used to quantify pediatric vascular reactivity. Because our focus is to improve the care of infants and children with neurological injury, we have performed our assessment in the spectral range of B waves, where the blood volume–ABP phase shift is complete. Because B waves were initially defined from ICP tracings in head-injured patients by Lundberg, we have favored the term slow waves to refer to vasogenic cycling between 0.004 and 0.05 Hz. It has been assumed that the irregularity in slow-wave frequency and amplitude is a disadvantage in analysis of vascular reactivity at the B-wave frequency. However, the growing body of outcome data with the PRx and our own animal data suggest that this assumption has more theoretical than real merit. To use slow waves for autoregulation monitoring in patients without traumatic brain injury will require demonstration of slow waves in the population targeted for monitoring. Slow vasogenic cycling has been documented in patients without head trauma such as septic patients, premature neonates, and healthy volunteers. Furthermore, in the present study, we have demonstrated slow waves in piglets at rest without intracranial or hemodynamic manipulation (Figures 2 and 3). PRx and HVx were observed to increase in grades as CPP was reduced below the LLA. This finding implies that the vasculature is not completely passive at the breakpoint of CBF autoregulation and is consistent with the concept that additional vasodilation occurs below the LLA. However, this additional vasodilation is insufficient to maintain CBF.

Clinical considerations for the HVx are prompted by the convenient and relatively noninvasive nature of the measurement. The HVx could possibly be rendered completely noninvasive by the use of noninvasive ABP monitoring with the Finapres device. The PRx has long been the only continuous assessment of vascular reactivity, and it requires an ICP monitor. Our objective in rendering a near-infrared-based vascular reactivity index was to develop a tool that can be used to define the bounds of autoregulatory reserve when the ICP monitor is unavailable. In particular, we seek to meet the pressing need to define autoregulation boundaries in infants and children that have yet to be satisfactorily explored. The pediatric population has an elevated incidence of, and mortality from, traumatic brain injury and is a subset of patients for whom we lack data to formulate even basic perfusion pressure goals. Premature infants have a risk of germinal matrix hemorrhage and periventricular ischemia, but there is no standard practice of neuromonitoring to guide the hemodynamic management of these unstable patients. It is possible that monitoring and optimizing vascular reactivity within ischemically sensitive regions of the premature brain might afford neuroprotection. With the HVx, this hypothesis can be tested without an ICP monitor.
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