

# Association between Endothelial Dysfunction and Acute Brain Dysfunction during Critical Illness

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## What We Already Know about This Topic

- Acute brain dysfunction manifested as delirium and coma is frequent during critical illness, but its pathophysiology remains unclear
- Endothelial function is impaired in critical illness and might contribute to altered cerebral blood flow and blood-brain barrier permeability

## What This Article Tells Us That Is New

- Endothelial function was assessed in a prospective cohort study of critically ill patients, and its relationship with acute brain dysfunction, assessed using multivariable linear regression
- Patients with worse systemic endothelial function had increased duration of acute brain dysfunction

## ABSTRACT

**Background:** Acute brain dysfunction (delirium and coma) during critical illness is prevalent and costly, but the pathophysiology remains unclear. The relationship of acute brain dysfunction with endothelial function, which is impaired in critical illness and may contribute to alterations in cerebral blood flow and blood-brain barrier permeability, has not been studied. This study sought to determine whether systemic endothelial dysfunction is associated with acute brain dysfunction during critical illness.

**Methods:** In this prospective cohort study, adult medical/surgical intensive care unit patients in shock and/or respiratory failure were enrolled. Endothelial function was assessed at enrollment using peripheral artery tonometry to calculate the reactive hyperemia index, with lower reactive hyperemia index indicative of worse endothelial function. Patients were assessed for coma and delirium with the Richmond Agitation-Sedation Scale and Confusion Assessment

◆ This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 9A. This article is accompanied by an Editorial View. Please see: Sanders RD: Delirium, neurotransmission, and network connectivity: The search for a comprehensive pathogenic framework. ANESTHESIOLOGY 2013; 118:494-6.

Method for the Intensive Care Unit. Multivariable linear regression was used to analyze the association between reactive hyperemia index and (1) delirium/coma-free days among all patients and (2) delirium duration among survivors, both over a 14-day period.

**Results:** One hundred forty-seven patients with median age of 57 yr and median Acute Physiology and Chronic Health Evaluation II score of 26 were enrolled. After adjusting for age, severity of illness, severe sepsis, preexisting cognitive function, medical *versus* surgical intensive care unit admission, and prehospital statin use, lower reactive hyperemia index (worse systemic endothelial function) was associated with fewer delirium/coma-free days ( $P = 0.02$ ) and more delirium days ( $P = 0.05$ ).

**Conclusions:** In this study, critically ill patients with lower vascular reactivity indicative of worse systemic endothelial function had increased duration of acute brain dysfunction.

**A**CUTE brain dysfunction (delirium and coma) during critical illness is highly prevalent and independently associated with prolonged mechanical ventilation, longer and more costly hospitalizations, increased risk of cognitive dysfunction months after hospital discharge, and higher mortality.<sup>1–7</sup> The leading factors of the pathogenesis of this acute brain dysfunction, such as inflammation, abnormal cerebral blood flow, and increased blood–brain barrier permeability,<sup>8,9</sup> involve the endothelium, a dynamic component of the blood–brain barrier with vasomotor and biochemical properties.

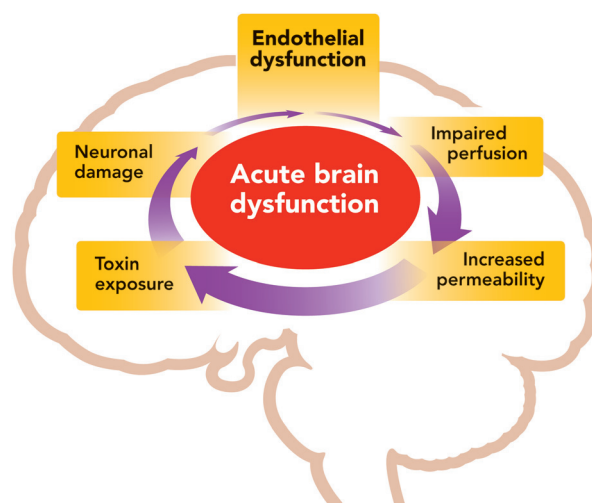
In critical illness states such as sepsis and multiple organ dysfunction syndrome, circulating inflammatory cytokines affect the endothelium's expression of adhesion molecules, signaling pathways, and nitric oxide production.<sup>10,11</sup> This further results in coagulation system activation, altered perfusion, distorted permeability, and decreased ability for vascular repair.<sup>12,13</sup> In the brain specifically, structural and functional alterations of blood–brain barrier endothelial cells secondary to inflammatory states have been associated with increased microvascular permeability and impaired microcirculatory blood flow.<sup>14–17</sup>

Given the relationship between endothelial dysfunction and potential mechanisms of brain dysfunction during critical illness, we hypothesized that critically ill patients with systemic vascular endothelial dysfunction would be at greater risk for acute brain dysfunction (fig. 1). To test this hypothesis, we assessed endothelial function *via* a reactive hyperemia technique with peripheral artery tonometry in medical and surgical intensive care unit (ICU) patients and prospectively assessed them for delirium and coma.

## Materials and Methods

### Study Design and Population

This prospective cohort study was nested within a larger observational study evaluating long-term cognitive impairment in survivors of critical illness. In a protocol



**Fig. 1.** Proposed relationship between systemic endothelial dysfunction and acute brain dysfunction. Systemic endothelial dysfunction may contribute to impaired cerebral perfusion, increased blood–brain barrier permeability, and exposure of the brain to toxic substances, causing neuronal damage and further brain organ injury. This may present clinically as acute brain dysfunction (delirium and coma).

approved by the Vanderbilt University Institutional Review Board (Nashville, Tennessee), critically ill adults consecutively admitted to the medical or surgical ICU at Vanderbilt University Medical Center with respiratory failure and/or shock were considered for inclusion in this study. Exclusion criteria for the parent study included severe cognitive or neurodegenerative disease that prevented independent living at baseline, ICU admission for neurological insult or postcardiopulmonary resuscitation with suspected anoxic brain injury, active substance abuse or psychotic disorder, blindness, deafness, inability to speak English, moribund with life expectancy less than 24 h, onset of the current episode of respiratory failure or shock greater than 72 h before enrollment, and cardiac surgery within the past 3 months. Additional exclusion criteria for this study of endothelial function included upper extremity injury, presence of arteriovenous fistula, prone positioning, and unavailability of equipment/technician within 24 h of enrollment. After obtaining informed consent from the patient or an authorized surrogate, baseline endothelial function and daily brain dysfunction monitoring were performed as detailed in the following sections.

### Endothelial Function Assessment

Systemic endothelial function was assessed within 24 h of study enrollment by a reactive hyperemia technique using peripheral artery tonometry *via* the Endo\_PAT device (Itamar Medical Ltd, Franklin, MA) to determine vascular reactivity. Originally validated against coronary angiography,<sup>18,19</sup> the Endo\_PAT has also been used to evaluate endothelial function in patients with coronary

artery disease, atherosclerosis, hypertension, congestive heart failure, schizophrenia, sepsis, and many other disease processes.<sup>20–25</sup>

With the participant lying supine in bed with both hands located at the level of the heart, a pneumatic finger probe was placed on each hand to perform a plethysmographic recording of the finger arterial pulse wave amplitude. Baseline pulse wave amplitude measurements were recorded for 5 min. A blood pressure cuff was then inflated on one arm to a suprasystolic pressure (50 mmHg above systolic pressure), and complete occlusion of the tonometry signal was confirmed to minimize error from inaccurate pretest blood pressure readings. The other arm remained unoccluded to serve as the reference. Five minutes after the cuff was inflated, it was released to induce reactive hyperemia, and pulse wave amplitude was measured for an additional 5 min. With this technique, the reactive hyperemia index (RHI) is calculated as the ratio between the magnitude of the average postocclusive pulse wave amplitude and the average baseline pulse wave amplitude, corrected to systemic changes seen in the unoccluded arm. The Endo\_PAT measurements and calculations use a computerized algorithm that requires no specific input from the technician, thereby reducing observer variability. A lower RHI indicates worse endothelial function, with values less than 1.67 considered to represent endothelial dysfunction.<sup>18,19</sup> The Endo\_PAT technician was blinded to results of brain dysfunction assessments done by research personnel, and the research personnel performing brain dysfunction assessments were blinded to RHI results.

### Brain Dysfunction Monitoring

A trained research nurse or assistant assessed every patient for acute brain dysfunction (coma and delirium) twice per day in the ICU and once per day in the ward using the Richmond Agitation–Sedation Scale and Confusion Assessment Method for the ICU, as per our standardized online §§ manual.<sup>2,26</sup> Assessments were performed until death, hospital discharge, or 14 days postenrollment, whichever occurred first. By definition, patients were considered delirious if they were not comatose (*e.g.*, had a Richmond Agitation–Sedation Scale score of –3 or more awake) and were Confusion Assessment Method for the ICU evaluation. Coma was defined as a Richmond Agitation–Sedation Scale score of –4 (responsive to physical stimulus only) or –5 (completely unresponsive).

### Statistical Analysis

To effectively examine the association between systemic endothelial dysfunction and acute brain dysfunction while accounting for bias due to death, we used the number of days alive and without delirium or coma (*e.g.*, delirium/coma-free days) during the 14 days after study enrollment as our primary outcome (dependent variable). Patients

who were discharged from the hospital before study day 14 were assumed to be nondelirious for days after discharge. To specifically examine the association between endothelial dysfunction and delirium, we used a secondary outcome of duration of delirium during this same 14-day period. We designated this a secondary analysis *a priori* and included only survivors because early death would curtail delirium duration and bias results of this analysis.

We used multivariable linear regression to assess the independent association of systemic endothelial function, as assessed by Endo\_PAT RHI, with the primary and secondary outcomes, after adjusting for relevant covariates. These covariates were chosen *a priori* based on prior research and clinical judgment and included age, severity of illness as measured by acute physiology score of the Acute Physiology and Chronic Health Evaluation II<sup>27</sup> score, severe sepsis at ICU admission defined as known or suspected infection with two or more systemic inflammatory response syndrome criteria and presence of organ dysfunction (mechanical ventilation and/or vasopressor requirement), preexisting cognitive function as measured by the Informant Questionnaire on Cognitive Decline in the Elderly<sup>28</sup> score, medical *versus* surgical ICU admission, and prehospital statin use. Delirium/coma-free days have a bimodal distribution and, as such, present a challenge in statistical modeling. Our biostatisticians (Thompson and Dr. Shintani), therefore, performed simulations to assess the suitability of using linear regression, proportional odds logistic regression, and several other strategies to model delirium/coma-free days. The results of the simulations showed that linear regression was performed reliably and was as powerful as any method. Given these results, and the fact that many clinicians are familiar with linear regression, we have chosen to use the same as our primary analysis. All continuous variables, including Endo\_PAT RHI, were initially allowed to have a nonlinear relationship with the outcome, using restricted cubic splines. If there was evidence that an association was nonlinear, (*e.g.*, the *P* for nonlinearity was lesser than 0.20), the nonlinear term was retained in the model; otherwise, the nonlinear terms were removed for parsimony. We chose to report the adjusted difference in outcomes between the 25th and 75th percentile Endo\_PAT RHI values of our population rather than the difference in outcomes per each single-unit change in Endo\_PAT RHI given that single-unit changes in vascular reactivity endothelial function assessments are not clinically meaningful. We used R version 2.15.1 for all statistical analyses.<sup>29</sup>

The sample size for this nested cohort was based on the primary outcome of days alive and without delirium or coma, modeled using multivariable linear regression with the variables listed in the previous paragraph. Continuous variables required two degrees of freedom for nonlinearity, whereas dichotomous variables required one degree of freedom. Therefore, the minimum degrees of freedom required for the model was 11. Assuming that one degree of freedom required 15 patients to reliably fit the model, a multivariable

§§ www.ICUdelirium.org. Accessed June 5, 2012.

model with a complexity of 11 degrees of freedom requires an effective sample size of  $11 \times 15 = 165$  subjects.<sup>30</sup>

## Results

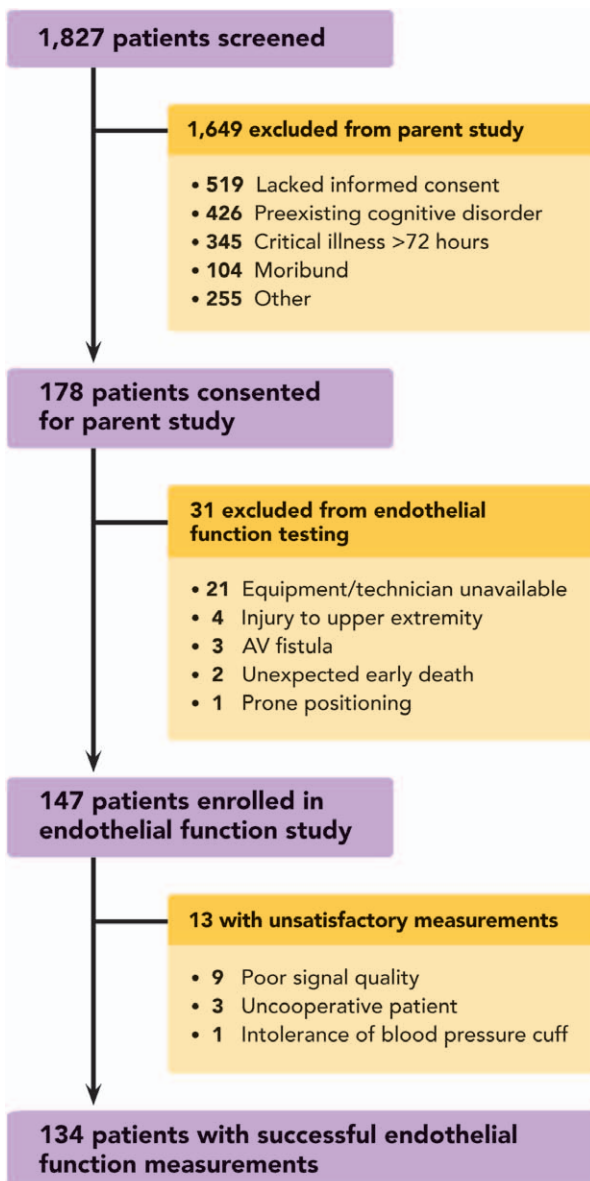
From April 2009 to September 2010, 178 patients were consented and enrolled into the parent study (fig. 2). Thirty-one of these patients met additional exclusion criteria for peripheral artery tonometry endothelial function assessment. As a result, 147 patients participated in endothelial function assessments in this study.

Patient characteristics and outcomes are presented in table 1, which indicate that the study population had a high severity of illness, with a median Acute Physiology and Chronic Health Evaluation II score of 26 and frequent organ

dysfunctions. Additionally, 30% of patients had severe sepsis at admission, and 85% were mechanically ventilated at study enrollment. Acute brain dysfunction was also common, with 70% of patients having delirium at some point during the 14-day study period. The median duration of delirium was 2 days, and the median duration of coma was 1 day.

Systemic endothelial function was successfully assessed by Endo\_PAT RHI in 134 patients who had a median (interquartile range) RHI of 1.51(1.32–1.81). For 13 patients who were assessed with the Endo\_PAT, the device was unable to provide satisfactory RHI evaluations (fig. 2). There were no significant differences in the demographic data between patients in whom RHI was *versus* was not successfully measured.

After adjusting for age, severity of illness, severe sepsis at admission, preexisting cognitive function, medical *versus* surgical ICU admission, and prehospital statin use, worse systemic endothelial function—indicated by a lower RHI as measured by the Endo\_PAT—was independently associated with fewer delirium/coma-free days ( $P = 0.02$ ; table 2 and fig. 3). Other covariates being equal, for example, a patient with a RHI of 1.32 (the 25th percentile in our study population) would have, on average, 0.8 fewer delirium/coma-free days during the 14-day study period (95% CI:  $-1.54, -0.12$ ;  $P = 0.02$ ) than a patient with a RHI of 1.81 (the 75th percentile). Worse systemic endothelial function was also marginally associated with increased duration of delirium in the



**Fig. 2.** Diagram of study profile. Prospective cohort nested within a larger observational study evaluating long-term cognitive impairment after critical illness. AV = arteriovenous.

**Table 1.** Demographic Characteristics and Outcomes of the Study Population

Variable*	N = 147
Age, yr	57 (47, 67)
Males, %	56
APACHE II at enrollment	26 (19, 31)
Severe sepsis at admission, %	30
IQCODE at enrollment	3 (3, 3.2)
Prehospital statin use (yes, %)	32
ICU type	
Medical ICU, %	54
Surgical ICU, %	46
Reactive hyperemia index	1.51 (1.32, 1.81)
Mechanically ventilated at enrollment, %	85
Duration of mechanical ventilation, days	3 (0.9, 7.1)
ICU length of stay, days	4.7 (2.0, 11.1)
Hospital length of stay, days	8.9 (5.2, 17.1)
Delirium present during 14-day study period, %	70
Delirium duration, days	2 (0, 4)
Coma duration, days	1 (0, 4)
Delirium/coma-free days†	10 (3, 13)

\* Median (interquartile range) unless specified. † The number of days alive and without delirium or coma during the 14 days after study enrollment.

APACHE = Acute Physiology and Chronic Health Evaluation<sup>27</sup>; ICU = intensive care unit; IQCODE = Informant Questionnaire on Cognitive Decline in the Elderly.<sup>28</sup>



**Table 2.** Endothelial Dysfunction and Brain Dysfunction: Delirium/Coma-Free Days

N = 134	25th Percentile	75th Percentile	Adjusted Difference 25th vs. 75th (95% CI)*	P Value
Independent variable				
Reactive hyperemia index	1.32	1.81	−0.8 (−1.54, −0.12)	0.02
Covariates				
Age, yr	47	67	1.2 (0.03, 2.37)	0.05
APACHE APS	17	27	2.2 (1.11, 3.35)	<0.001†
ICU type	Medical	Surgical	0.5 (−1.34, 2.28)	0.61
IQCODE	3.00	3.19	0.1 (−0.42, 0.53)	0.83
Prehospital statin use	No	Yes	−0.4 (−2.29, 1.46)	0.66
Severe sepsis	No	Yes	1.2 (−0.87, 3.18)	0.27

Endothelial function, the independent variable, was assessed using the Endo\_PAT (Itamar Medical Ltd, Franklin, MA) reactive hyperemia index, a validated assessment of systemic endothelial function where lesser than 1.67 is considered endothelial dysfunction.<sup>18,19</sup> Linear regression was used to study the association of endothelial function (N = 134) with acute brain dysfunction after adjusting for age, acute physiology score of the Acute Physiology and Chronic Health Evaluation II,<sup>27</sup> severe sepsis, Informant Questionnaire on Cognitive Decline in the Elderly<sup>28</sup> score, medical vs. surgical ICU admission, and prehospital statin use. The adjusted difference represents the difference in delirium/coma-free days between the 25th vs. the 75th percentile for continuous variables or no vs. yes for dichotomous variables. Interpretative example: Other covariates being equal, patients with a reactive hyperemia index at the 25th percentile (1.32, poor endothelial function) would have, on average, 0.8 fewer delirium/coma-free days than patients with a reactive hyperemia index at the 75th percentile (1.81, good endothelial function).

\* In this analysis, a positive value is indicative of better outcomes. † Nonlinear relationship.

APACHE = Acute Physiology and Chronic Health Evaluation;<sup>27</sup> APS = Acute Physiology Score; ICU = intensive care unit; IQCODE = Informant Questionnaire on Cognitive Decline in the Elderly.<sup>28</sup>

113 survivors ( $P = 0.05$ ; table 3 and fig. 4). Similarly, as above, a patient with a RHI of 1.32 (25th percentile) would have, on average, 0.4 more days of delirium during the study period (95% CI: 0.00, 0.86;  $P = 0.05$ ) than a patient with a RHI of 1.81 (75th percentile).

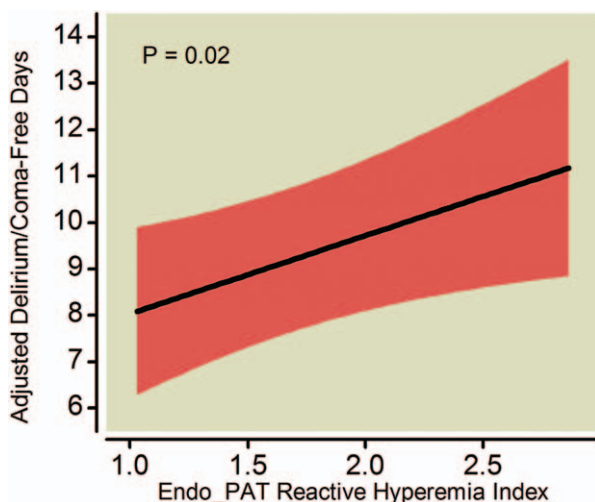
In addition to worse endothelial function, older age and higher severity of illness (measured by the acute physiology

score of the Acute Physiology and Chronic Health Evaluation II score) were also independently associated with fewer delirium/coma-free days in all patients (table 2) and increased delirium duration in survivors (table 3), consistent with previous studies.<sup>31–33</sup> A sensitivity analysis adjusting for underlying vascular disease, as defined by the Framingham Stroke Risk Profile,<sup>34</sup> still found a significant association between Endo\_PAT RHI and delirium/coma-free days ( $P = 0.04$ ), although the association with delirium days was marginal ( $P = 0.06$ ).

## Discussion

In this study, the first study to examine the potential relationship of systemic endothelial dysfunction and acute brain dysfunction during critical illness, we found that impaired vascular reactivity indicative of worse systemic endothelial function was independently associated with fewer delirium/coma-free days. This association is consistent with the hypothesis that endothelial dysfunction plays a role in the pathogenesis of acute brain dysfunction during critical illness. Thus, this investigation should prompt further studies to examine mechanisms whereby endothelial dysfunction may contribute to the pathogenesis of brain dysfunction in the critically ill.

Despite increasing research in the field, the complex pathophysiology of acute brain dysfunction in the critically ill remains elusive. Several hypotheses have been developed, with many centering on the effects of inflammation in the brain.<sup>35,36</sup> The hypothesis we propose herein, that endothelial dysfunction contributes to acute brain dysfunction during critical illness, is not an alternative but rather a complement



**Fig. 3.** Endothelial function versus delirium/coma-free days. Worse systemic endothelial function at enrollment (indicated by a lower Endo\_PAT reactive hyperemia index, where less than 1.67 is considered endothelial dysfunction) was associated with fewer delirium/coma-free days among all patients after adjusting for covariates. The black line demonstrates the point estimate of the association between reactive hyperemia index and delirium/coma-free days, with the red ribbon indicating the 95% CI.

**Table 3.** Endothelial Dysfunction and Brain Dysfunction: Delirium Duration in Survivors

N = 113	25th Percentile	75th Percentile	Adjusted Difference 25th vs. 75th (95% CI)*	P Value
Independent variable				
Reactive hyperemia index	1.32	1.81	0.4 (0.00, 0.86)	0.05
Covariates				
Age, yr	47	67	-1.5 (-2.46, -0.51)	0.01†
APACHE APS	17	27	-1.0 (-1.66, -0.24)	0.008†
ICU type	Medical	Surgical	-1.1 (-2.33, 0.06)	0.06
IQCODE	3.00	3.19	0.1 (-0.23, 0.35)	0.71
Prehospital statin use	No	Yes	-0.2 (-1.44, 1.01)	0.73
Severe sepsis	No	Yes	-0.6 (-1.95, 0.67)	0.34

Endothelial function, the independent variable, was assessed using the Endo\_PAT (Itamar Medical Ltd, Franklin, MA) reactive hyperemia index, a validated assessment of systemic endothelial function where less than 1.67 is considered endothelial dysfunction.<sup>18,19</sup> Linear regression was used to study the association of endothelial function with duration of delirium in patients surviving to 14 days (N = 113) after adjusting for age, acute physiology score of the Acute Physiology and Chronic Health Evaluation II,<sup>27</sup> severe sepsis, Informant Questionnaire on Cognitive Decline in the Elderly<sup>28</sup> score, medical vs. surgical ICU admission, and prehospital statin use. This analysis was limited to survivors to avoid bias due to death. The adjusted difference represents the difference in delirium duration between the 25th and 75th percentile for continuous variables or no vs. yes for dichotomous variables. Interpretative example: Other covariates being equal, patients with a reactive hyperemia index at the 25th percentile (1.32, poor endothelial function) would have, on average, 0.4 more days with delirium than patients with a reactive hyperemia index at the 75th percentile (1.81, good endothelial function).

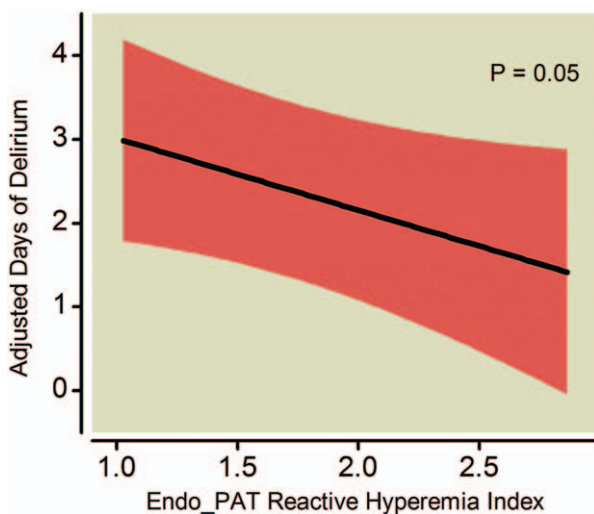
\*In this analysis, a negative value is indicative of better outcomes. †Nonlinear relationship.

APACHE = Acute Physiology and Chronic Health Evaluation;<sup>27</sup> APS = Acute Physiology Score; ICU = intensive care unit; IQCODE = Informant Questionnaire on Cognitive Decline in the Elderly.<sup>28</sup>

to the inflammation hypothesis of acute brain dysfunction during critical illness. In sepsis, the prototypical example of an inflammatory state during critical illness, inflammatory mediators have been shown to bind directly to receptors on the endothelium, leading to cell detachment, cell death, altered vascular function, microvascular injury, and altered organ perfusion.<sup>12,13</sup> In the brain, reduced blood

flow and increased vascular permeability during sepsis lead to neuronal inflammation and tissue damage, modulating synthesis of neurotransmitters and altering expression of neurotransmitter receptors.<sup>35–37</sup> Given that the blood–brain barrier consists of endothelial cells, along with astrocyte foot processes, it is plausible that patients with endothelial dysfunction may have an exaggeration of such responses. Altered endothelial vasomotor function and increased expression of adhesion molecules and coagulation mediators from endothelial activation may lead to altered perfusion, distorted permeability, thrombosis, and decreased ability for repair that contribute to the development of brain organ dysfunction. If so, a reliable measure of systemic endothelial function may act as a noninvasive surrogate for blood–brain barrier function involved in brain dysfunction during critical illness.

Reactive hyperemia is a noninvasive technique to assess endothelial function. During reactive hyperemia, endothelial nitric oxide release mediates changes in vascular tone and blood flow. Using validated tools, such as peripheral artery tonometry and brachial artery reactivity testing, investigators and clinicians can measure these changes in vascular reactivity and thereby assess endothelial function at the bedside.<sup>18,19,38</sup> Attenuations in reactive hyperemia and other endothelial-dependent dilator responses indicate endothelial dysfunction and have been associated with poor cardiovascular and cerebrovascular outcomes, severity of sepsis, and mortality during critical illness.<sup>20,39–43</sup> In previous studies, systemic endothelial function has been evaluated using several methods, including flow-mediated dilation of the brachial artery as a response to reactive hyperemia, aortic augmentation index requiring arterial cannulation and vasodilator administration, and laser



**Fig. 4.** Endothelial function *versus* delirium duration in survivors. Worse systemic endothelial function at enrollment (indicated by a lower Endo\_PAT reactive hyperemia index, where less than 1.67 is considered endothelial dysfunction) was associated with more days of delirium in survivors after adjusting for covariates. The *black* line demonstrates the point estimate of the association between reactive hyperemia index and days of delirium, with the *red* ribbon indicating the 95% CI.

Doppler plethysmography also requiring vasodilator administration.<sup>39,43,44</sup> Because these methods are invasive or require skilled operators, we chose to use the Endo\_PAT device, which relies on peripheral artery tonometry to calculate the RHI, a validated assessment of endothelial function. The device is easy to use, noninvasive, portable, and uses a computerized algorithm to assess function.

The results of our study are consistent with those of previous studies showing that endothelial function is worse in the setting of disease and acute illness. Although the majority of healthy patients with few or no cardiac risk factors have average RHI values greater than 2.2, those with coronary artery disease (2.0), sepsis without organ failure (1.85), and sepsis with organ failure (1.57) show progressively worsening endothelial function.<sup>20,25</sup> Our cohort, consisting of ICU patients with shock and/or respiratory failure, had a median RHI of 1.51, suggesting even poorer endothelial function than that previously reported. The results of our study, along with those of Davis *et al.*,<sup>20</sup> support that endothelial function assessments with the Endo\_PAT can be performed at the bedside in critically ill patients. Thus, if future studies confirm that Endo\_PAT RHI measurements are associated with patients' acute cognitive outcomes, endothelial function monitoring may serve as a prognostic tool in the ICU as well as a means to study the impact of therapies aimed at reducing brain dysfunction through interventions that enhance endothelial function (*e.g.*, statin pharmacotherapy, early physical rehabilitation).<sup>45</sup>

Our results are also in agreement with the growing body of literature examining tissue perfusion abnormalities as measured by near-infrared spectroscopy. Consistently low tissue oxygenation was associated with worse organ failure in small cohorts of critically ill patients,<sup>46</sup> and lower tissue oxygen saturation and slower recovery rate after an ischemic period has been correlated to perfusion abnormalities.<sup>47</sup> In addition, abnormal tissue oxygenation recovery after an ischemic period has been demonstrated in patients with sepsis and is more pronounced in patients with septic shock.<sup>48,49</sup> Data with regards to brain dysfunction are still evolving, as cerebral tissue oxygenation was not associated with sepsis-associated delirium,<sup>50</sup> but lower preoperative cerebral tissue oxygenation was associated with postoperative delirium in another small cohort study.<sup>51</sup>

Our study has several strengths and limitations. Since many conditions (*e.g.*, genetic makeup, comorbid diseases including previous vascular disease, current critical illness) affect the endothelium, we examined the culmination of these effects by assessing endothelial function at enrollment. Importantly, we found the association between endothelial function and duration of brain dysfunction to remain significant even when vascular comorbid disease burden was included in the sensitivity analysis. We relied on a functional assessment of systemic endothelial vasomotor performance (rather than a static lab value of endothelial injury) and showed that active endothelial dysfunction was associated

with worse brain dysfunction outcomes, independent of age, severity of illness, or vascular risk factors. It is possible that factors associated with critical illness (*e.g.*, disease processes, therapeutics) might differently affect endothelial vasomotor function and endothelial injury resulting in biomarker release, an area of particular interest in the pathophysiology of inflammatory disease states such as sepsis.<sup>52,53</sup>

The single-center design may limit generalizability to only patient populations similar to ours; however, we enrolled a heterogeneous population by including medical and surgical ICU patients with a broad range of diagnoses. Our study is also the largest to use the Endo\_PAT device to assess endothelial function in critically ill patients. Poor signal quality for peripheral artery tonometry prevented RHI measurement during only 9 (6%) of 147 studies performed, despite the majority of patients receiving sedatives, vasopressors, and/or mechanical ventilation during their evaluation. Lack of patient cooperation prevented another three RHI measurements, raising the question of whether patients with hyperactive delirium can be assessed with the Endo\_PAT; this scenario was very rare, however, in keeping with studies showing that pure hyperactive delirium is uncommon in critically ill patients.<sup>54</sup> Upper extremity defects or prone positioning also prevented the performance of peripheral artery tonometry, but this was rare; however, radial arterial catheters which are common in the ICU did not affect the ability to obtain RHI measurements. Given the pilot nature of this study, the first to examine the association between systemic endothelial function and acute brain dysfunction, we only assessed endothelial function at one time point (enrollment) to test our hypothesis. Serial assessments would have provided an indication of how endothelial alterations evolve during critical illness and in patients with acute brain dysfunction and should be considered in future studies.

Although our study was larger than previous investigations of endothelial vasomotor function during critical illness, a larger sample size would enhance statistical power and allow the inclusion of additional potential confounders, such as specific comorbidities, inflammatory mediators, and exposure to vasoactive or sedative medications. When required, vasopressor agents were administered to patients *via* central venous access, thus causing systemic (not local) effects, which are accounted for in the RHI calculations by inclusion of baseline pulse wave amplitudes and pulse wave amplitudes from the reference unobstructed arm. Measurements were also taken during steady-state infusion and not during drug bolus. Furthermore, previous research suggests that the RHI itself is unlikely to be significantly altered by vasopressor administration.<sup>55–57</sup> Future studies examining the role of endothelial dysfunction in brain dysfunction outcomes, however, need to evaluate potential mechanisms of mediation, including inflammation, markers of endothelial injury, and therapeutics such as sedation.



## Conclusion

In this prospective cohort study of severely ill medical and surgical ICU patients, we found that impaired vascular reactivity indicative of worse systemic endothelial function was independently associated with acute brain dysfunction. Although our study was not intended to and cannot show a mechanistic relationship between endothelial dysfunction and acute brain dysfunction, our findings lend support to the hypothesis that endothelial dysfunction plays a role in the pathogenesis of acute brain dysfunction during critical illness. In light of these findings, subsequent investigations are needed to examine specific mechanisms (*e.g.*, disruption of the blood–brain barrier or cerebral blood flow), whereby endothelial dysfunction may contribute to the pathogenesis of brain dysfunction during critical illness. Such studies could point to therapies that modify endothelial function and thereby improve short- and long-term neurologic outcomes in critically ill patients.

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