Endothelial Cell Function and Dysfunction in Critically Ill Children

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The vascular system is a continuous organ reaching to within micrometers of all tissue parenchymal cells. Endothelial cells (ECs) continuously line the lumen of this system and function as follows: to regulate blood flow; maintain blood fluidity; control fluid, solute, and macromolecular transfer between blood and tissue; and modulate circulating immune cell recruitment and activation. These vital functions, combined with the broad anatomic distribution of ECs, implicate them in all forms of critical illness. The present article discusses how ECs adapt and break down during the course of critical illness. We first review the biology of ECs, highlighting the vascular segmental differences and their specific roles in the maintenance of homeostasis. We then discuss how ECs acquire new functions to restore local and systemic homeostasis (activation) as well as how breakdowns in EC functions (dysfunction) contribute to local and systemic pathologic responses, with clinical correlations. Lastly, how these processes have been studied in critically ill children is discussed.

Endothelial cells (ECs) line the lumen of the entire vascular system and actively regulate blood flow; maintain blood fluidity; control water, solute, and macromolecular transfer between blood and tissue; and modulate circulating immune cell recruitment and activation. These vital functions, combined with the broad anatomic distribution of ECs, implicate them in all forms of critical illness. The present article discusses how ECs adapt and break down during the course of critical illness. We first review the biology of ECs, highlighting the vascular segmental differences and their specific roles in the maintenance of homeostasis. We then discuss how ECs acquire new functions to restore local and systemic homeostasis (activation) as well as how breakdowns in EC functions (dysfunction) contribute to local and systemic pathologic responses, with clinical correlations. Lastly, how these processes have been studied in critically ill children is discussed.

EC SEGMENTAL DIFFERENCES

The circulatory system comprises 3 distinct segments: arteries, microvessels (discussed in the following sections), and veins. In all vascular segments, ECs are attached to an underlying basement membrane comprising primarily type IV collagen, laminin, and glycosaminoglycans. The EC luminal surface projects a glycocalyx coating containing heparan-sulfate proteoglycans that serve as binding sites for multiple proteins (eg, coagulation factors, ectoenzymes, chemokines). Arteries conduct blood to each organ bed and are continuously lined by ECs connected to each other by tight junctions, forming largely impermeable barriers that produce minimal change in vascular volume during systolic pressure wave conduction. Distal to the microvasculature, the elastic veins have larger lumens and serve as capacitance (storage) reservoirs for up to three-quarters of total
blood volume. ECs lining veins are interconnected principally by adherens junctions, which can minimize fluid passage under low-pressure conditions.  

The microvasculature, composed of arterioles, capillaries, and venules, is highly variable in both structure and function, in a manner that accommodates each organ-specific microenvironment. Larger arterioles, similar to arteries, are supported by circumferentially arranged layers of smooth muscle cells (SMCs), whereas the terminal precapillary arteriolar segments are supported by pericytes that reside within a basement membrane shared with ECs (Fig 1A). The precapillary arteriolar endothelium serves primarily to regulate capillary perfusion and recruitment in different organ beds. Capillary ECs vary in the number of tight junctions and the extent of their overlap with neighboring cells, ranging from essentially impermeable in the central nervous system to profoundly leaky in splenic or hepatic sinusoids. Although capillaries are individually small, with lumens <10 µm, they are exceedingly numerous and their massive cumulative surface area is the primary site for gas and metabolite exchange. In most tissue, the EC lining of the capillaries is continuous, and exchange of macromolecules is generally restricted by capillary ECs to specialized cellular structures such as fenestrae or vesicles.1 The glycocalyx coating of the ECs, although present in all segments, is particularly important in the capillaries because it occupies a substantial proportion of the lumen. The capillary wall tends to be simpler than elsewhere in the vasculature, consisting of an EC monolayer supported by adherent pericytes. The ratio of pericytes to ECs varies widely, from 1:1 in the central nervous system to <1:10 in certain peripheral beds. In certain regions of the vasculature, pericytes are further specialized (ie, mesangial cells in the renal glomeruli and stellate cells in the sinusoids of the liver). In the postcapillary venular segment, ECs are mostly devoid of tight junctions, being interconnected largely by adherens junctions. Consequently, the postcapillary venules are usually intrinsically leakier than the capillaries. The venules are also the first site of physiologic leukocyte recruitment and increased permeability in the setting of localized inflammation.

**EC FUNCTIONS IN HOMEOSTASIS**

No longer viewed as merely a passive lining, ECs are now understood to play active roles in many functions essential for homeostasis. ECs may be activated by local stimuli, acquiring new functions intended to restore homeostasis.7 These acquired functions are unique to specific organs and reflect functional specialization of ECs.

**Blood Flow**

ECs generate vasodilating and vasoconstricting factors that act on SMCs and pericytes both locally and systemically. ECs constitutively express nitric oxide (NO) synthase-3, which is activated by shear forces sensed by the ECs, producing a steady, low basal level of NO (Fig 1A). Blocking NO synthase-3 raises blood pressure, identifying this factor as an important basal function.8 In addition, many local or system mediators may act on ECs to stimulate NO production via a calcium-calmodulin activation pathway. NO diffuses from ECs into surrounding SMCs, where it activates soluble guanylate cyclase to produce cyclic guanosine monophosphate, leading to SMC relaxation and increased lumen caliber. In addition, ECs constitutively express cyclooxygenase-1 and, when activated, may express cyclooxygenase-2. These enzymes convert arachidonic acid, liberated from the plasma membrane by calcium-activated cytosolic phospholipase A₂ to prostaglandin H₂, which is then converted to prostaglandin I₂ or prostaglandin E₂. These lipid mediators act on SMCs to produce relaxation and vasodilation by increasing intracellular cyclic adenosine monophosphate levels.

In some circumstances, EC-generated arachidonic acid can alternatively be converted to thromboxane-A₂, inducing SMCs to elevate cytosolic calcium ion producing vasoconstriction. ECs may also produce endothelin-1 (ET-1) by synthesis of its propeptide, big-endothelin, and expression of endothelin-converting enzyme on their luminal surface. ECs also express angiotensin-converting enzyme that produces angiotensin-II (ATII) from EC-secreted angiotensin-I. Both ET-1 and ATII act as potent vasoconstrictors by increasing SMC cytosolic calcium levels. These processes are targeted in therapies for pulmonary hypertension in children and neonates. Increasing cyclic guanosine monophosphate or cyclic adenosine monophosphate levels in SMCs is achieved with inhaled NO and phosphodiesterase-5 inhibitors or stable prostacyclin analogues, respectively. The endothelin pathway is targeted with endothelin receptor antagonists, whereas ATII may be targeted either by inhibitors of angiotensin-converting enzyme or of type 1 angiotensin receptors. In general, capillary activation is characterized by increasing microperfusion by either increasing flow through open vessels or recruiting unperfused collateral capillaries mediated by an NO feedback system involving red blood cells.9

**Hemostasis**

All vascular segments maintain blood fluidity via prevention of thrombus
and the endothelium directly controls the reflection coefficient ($K_f$). Flux of fluid is governed by the capillary and interstitial hydrostatic and oncotic pressures ($P_c$, $P_i$). Pericytes lining venular segments do not typically form tight junctions and are held together by adherens junctions, characterized by vascular cytoskeleton and closely apposed to the plasma membranes of adjacent cells, thus preventing paracellular passage of macromolecules ($MM$). Gases diffuse freely. Pericyte lining of venular segments do not typically form tight junctions and are held together by adherens junctions, characterized by claudin-5, junctional adherens molecules, and occluding-forming transmembrane bands of protein. These proteins directly link to the endothelial cell--cadherin and are much more permeable to passive solute and fluid flow allowing passive reabsorption of water into the vascular space. Platelet adhesion is decreased by EC masking of collagen in the basement membrane and by secretion of proteases ADAMTS-13 and -18 to reduce ultra-large vWF (UL-vWF) to polymers of a more physiologic size and activity. Ectoenzymes (EE) convert platelet-activating adenosine triphosphate (ATP) and adenosine diphosphate (ADP) to adenosine monophosphate (AMP). Platelet activation is also inhibited by EC-secreted PGI2 and NO. Coagulation is actively inhibited by antithrombin-III (ATIII)-mediated inactivation of Xa and thrombin (Th). In addition, thrombomodulin (TM) binds thrombin and directs it to activate protein C. Tissue factor pathway inhibitor (TFPI) blocks tissue factor (TF) and Vila. Thrombolysis is activated by EC-secreted tissue plasminogen activator (t-PA) and urokinase (UK). Coagulation may be promoted by downregulating the aforementioned mechanisms as well as increased secretion of UL-vWF, expression of TF, secretion of microparticles that provide lipid and surface that binds TF and activates the coagulation cascade, and secretion of plasminogen activator inhibitor-1 (PAI-1) that blocks thrombolysis by tPA and UK. C. The arteriole and capillary vascular segments may be lined by a continuous layer of ECs, with overlapping borders forming tight junctions characterized by claudin-5, junctional adherens molecules, and occluding-forming transmembrane bands of protein. These proteins directly link to the cytoskeleton and closely apposed to the plasma membranes of adjacent cells, thus preventing paracellular passage of macromolecules (MM). Gases diffuse freely. EC lining of venular segments do not typically form tight junctions and are held together by adherens junctions, characterized by vascular endothelial--cadherin and are much more permeable to passive solute and fluid flow allowing passive reabsorption of water into the vascular space. The flux of fluid is governed by the capillary and interstitial hydrostatic and oncotic pressures ($P_c$, $P_i$, $\pi_c$, and $\pi_i$) as detailed by Starling’s law, and the endothelium directly controls the reflection coefficient ($K_f$). Pericyte-derived angiopoietin-1 (ANGPT1) activates TIE2 and stabilizes the EC barrier, whereas its natural antagonist EC-derived angiopoietin-2 (ANGPT2) blocks activation and destabilizes the EC barrier. D. Quiescence of immune cells is mediated by basal secretion of NO. Inflammatory cytokines stimulate ECs to recruit leukocytes by inducing expression of selectins, notably E-selectin (that mediates leukocyte tethering and rolling), secretion and display of chemokines (CX) on the glycoalyx (that activates leukocyte spreading and mobility), and expression of ligands for leukocyte integrins such as ICAM-1 and VCAM-1 all induced by the action of pro-inflammatory cytokines. ANGPT2 antagonizes TIE2 signaling, potentially increasing the effects of pro-inflammatory cytokine signaling. Release of membrane-bound ICAM produces soluble ICAM-1 (sICAM-1), a widely viewed marker of immune activation with undetermined physiologic impact.
thrombotic thrombocytopenic purpura is due to the development of anti-ADAMTS-13 antibodies that block its function. In addition, ECs display ectoenzymes that convert platelet-activating adenosine triphosphate and adenosine diphosphate to inactive adenosine monophosphate. ECs also prevent platelet activation via contact with the basement membrane collagen, whereas thrombin, another potent platelet activator, is inhibited by mechanisms described earlier. Finally, ECs participate in thrombolysis by synthesis and secretion of tissue-plasminogen activator and urokinase, promoting the conversion of plasminogen to plasmin.

Permeability

The endothelium is responsible for maintenance of intravascular volume by regulating the perselectivity of the vessel wall. Fluid flux ($J$) between the vascular and interstitial compartments is governed by pressures gradients as described by Starling’s equation, shown in Fig 1C. The filtration ($K_f$) and reflection ($\sigma$) coefficients are determined by local EC properties while the intracapillary blood and interstitial hydrostatic and oncotic pressures ($P_v, P_f, \pi_c$, and $\pi_i$, respectively) depend on more systemic factors. Although the arteries are generally impermeable, the organ-specific microenvironments dictate modifications of EC junctions, thereby altering $K_f$, resulting in local permeability changes, predominately in the capillary and venule segments. In the capillaries, the exchange of oxygen and carbon dioxide is passive, whereas the macromolecules are actively transported in transcellular vesicles, resulting in a perselective barrier in which extravasation or intravasation varies with molecular species. At the arteriolar side of the capillary segment, hydrostatic pressure drives fluid across the capillary EC lining, concentrating macromolecules in the vascular lumen; this action results in increased capillary oncotic pressure that drives fluid and solutes intravascularly toward the postcapillary venular segment, where the hydrostatic pressure is reduced. Impaired postcapillary fluid absorption resulting from reduced intracapillary blood oncotic pressure ($\pi_i$), due to nephrotic syndrome or other conditions in which plasma proteins are lost, may produce pitting edema in children. Capillary properties are tissue dependent; for example, the permeability of the blood–brain barrier is extremely limited with a high density of inter-EC tight junctions that restrict solutes and only allow free water to pass paracellularly. However, the hepatic sinusoids have gaps between adjacent ECs that may even leave basement membranes exposed, permitting passage of large proteins between hepatocytes and the blood stream.

Regulation of Inflammation

The venular endothelium primarily regulates the recruitment of leukocytes to areas of tissue damage or infection and modulates leukocyte activity in areas of inflammation (Fig 1D). Leukocyte recruitment is limited under basal conditions because EC-leukocyte interaction is prevented by absent or limited expression of leukocyte-binding adhesion molecules such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1). In response to localized inflammation, venular ECs increase their surface expression of adhesion molecules and express chemokines to locally recruit leukocytes. Inherited defects in either adhesion molecules or their receptors on leukocytes result in syndromes called leukocyte adhesion deficiency and classically present as a neonate with delayed umbilical cord separation. Unstimulated ECs also synthesize and display fewer chemokines to activate leukocyte adhesion and motility and basally release NO, which inhibits leukocyte activation.

EC Activation and Dysfunction in Pediatric Critical Illness

EC activation is characterized by the acquisition of new cellular functions to restore homeostasis after a perturbation such as an inflammatory stimulus. In contrast, EC dysfunction is defined as the loss or inappropriate exaggeration of cellular functions leading to pathologic changes. EC dysfunction occurs during excessively prolonged or intense systemic stimulation, and individual cells may progress along a spectrum from activation, dysfunction, and injury to death. The relationship between EC homeostasis, activation, dysfunction, and death is a nonsequential spectrum with considerable segmental and organ-specific variation, such that multiple processes may simultaneously occur in children during the acute phase of critical illness. Although EC dysfunction is believed to worsen disease in critically ill children, the nature and extent of the endothelial response remain poorly understood due to difficulty assessing EC dysfunction directly. Traditional measures of the macrocirculation, such as blood pressure or pulse quality, may poorly correlate with changes in the crude assessment of the microcirculation, such as capillary refill time (CRT). Consequently, studies have focused on indirect (surrogate) assessment, such as levels of serum proteins shed or secreted by activated or injured ECs. These biomarkers do not provide enough precise information to determine that exact degree of
altered function. Here we describe the role ECs play in pediatric critical illness.

**Endothelial Dysfunction and Blood Flow**

In the setting of local inflammation, arteriolar dilation due to mediators such as prostaglandin I$_2$ increases regional blood flow, accounting for the classic inflammatory features of redness (rubor) and warmth (calor). In the setting of systemic inflammation, endothelial dysfunction leads to perfusion defects, a hallmark of shock. The term hemodynamic coherence refers to jointly functioning or dysfunctional macrovascular and microvascular blood flow (e.g., normal blood pressure and normal CRT or low blood pressure and prolonged CRT). In critically ill children, hemodynamic coherence may break down, and macrovascular parameters may not reflect microvascular flow at the cellular level, classically illustrated by the low blood pressure and flash CRT state of toxic shock syndrome. Arteriolar EC dysfunction results in pathologic vasoregulation and maldistribution of blood flow manifest as flash or prolonged capillary refill. A large study of 600 noncritically ill children showed that even mild infections impaired macrocirculatory brachial artery flow. Dysregulated capacitance caused by venular dysfunction leads to inadequate preload and poor cardiac output.

Assessments of the effects of EC regulation on microvascular blood flow in sick children are challenging but may be possible with newer techniques, such as sidestream dark-field imaging. A case report of an infant with septic shock found improvement in arm capillary blood flow, as measured by using sidestream spectroscopy, with improvement in macrocirculatory measures. Sublingual capillary perfusion, a surrogate assessment of global microperfusion, is also disrupted in sepsis and Dengue shock. Correlating the results of these techniques with levels of soluble EC adhesion molecules is also revelatory. One study found microcirculatory dysfunction, as measured by using sublingual capillary density, negatively correlated with blood levels of E-selectin, ICAM-1, and VCAM-1. The investigators speculated that these markers of EC activation corresponded to their ability to regulate perfusion. Levels of ICAM-1 and VCAM-1 in patients with sepsis were also elevated in those who developed multiorgan dysfunction syndrome. Although these molecules are not specific for ECs, the results suggest that defects in EC–leukocyte interactions may disrupt EC regulation of blood flow in sepsis. Collectively, these data suggest that ECs are responsive to even minor inflammatory stimulation, partially explaining the variable correlation of microcirculatory and macrocirculatory changes.

**Endothelial Dysfunction and Hemostasis**

Intimately entangled with both inflammation and blood flow, ECs also tightly regulate coagulation (Fig 1B). This feature is particularly emphasized during sepsis, leading to rapidly changing clinically impactful coagulopathies. Paradoxically, consumption of coagulation factors or platelets may result in bleeding. ECs may increase platelet adhesion to the vessel wall through release of preformed ultra-large vWF polymers from Weibel-Palade bodies. Damaged or dying ECs initiate coagulation by expression of tissue factor and by provision of phosphatidylserine-rich microparticles that serve as a platform for assembly of coagulation factors. At the same time, ECs may lose their capacities to inhibit coagulation and platelet activation leading to microvascular thrombosis. ECs that are chronically exposed to inflammatory cytokines decrease thrombomodulin and NO synthase-3 expression in correlation with increased platelet activation. Damaged or exfoliated endothelium can no longer prevent platelet contact with basement membrane collagen. To date, studies have focused mainly on concentrations of soluble coagulation cascade participants whose functions are primarily regulated by ECs, most notably activated protein C (aPC). The extensive research on aPC in pediatric sepsis, from case reports to the seminal multicenter clinical trials (the open-label ENHANCE [Extended Evaluation of Recombinant Human Activated Protein C] and randomized controlled RESOLVE [Researching Sepsis and Organ Dysfunction in Children: A Global Perspective] trials), is summarized in Table 1. These findings highlight the importance of EC-mediated coagulopathies as a marker of disease severity in sepsis. However, the lack of benefit from administered aPC suggests that ECs have the ability to modulate blood viscosity via multiple independent and overlapping mechanisms.

In addition to sepsis, exposure to foreign surfaces, as occurs during cardiopulmonary bypass (CPB), may disrupt the ability of ECs to modulate blood fluidity and coagulation by altering systemic inflammatory mediator levels and function, including thrombin/antithrombin and thrombomodulin. In addition, nonpulsatility during CPB may activate or induce dysfunction in ECs. Both EC-derived tissue plasminogen activator and its endogenous inhibitor, plasminogen activator inhibitor-1, are increased after CPB, whereas the tissue plasminogen activator/plasminogen activator inhibitor-1 ratio is maintained in pulsatile CPB flow. The precise mechanisms by which
these disruptions occur remain unclear.

**Endothelial Dysfunction and Permeability**

Permselectivity and control of blood–tissue fluid, solute, and macromolecule exchange may be lost in critical illness, producing edema in virtually every organ, leading to impaired diffusion-based gas and nutrient exchange at the cellular level. In fact, a postmortem examination found increased organ weight in critically ill children, consistent with accumulation of interstitial fluid. Venular EC activation, occurring in response to localized cytokine stimulation, results in physiologic increased permeability, and the relatively low surface area of venules allows for localized inflammatory reactions with few detectable systemic effects. Conversely, permeability changes associated with capillary dysfunction are usually systemic, always pathophysiologic, and have profound effects on macrocirculatory parameters and organ function. Two studies suggest that capillary EC permeability is altered in acute respiratory distress syndrome (ARDS) due to breakdown of pulmonary capillary tight junctions. In addition, there is decreased expression of vascular endothelial–cadherin, an adherens junction protein found in all vascular segments, in patients with ARDS. In children, capillary leak lacks a consensus definition or definitive testing, leading to diagnostic uncertainty, although these studies implicate its importance in pediatric critical illness.

The angiopoietin-TIE2 axis may play a role in both EC-mediated permeability and inflammation in critically ill children. Angiopoietin-1 (ANGPT1) is constitutively produced by pericytes and is a barrier-stabilizing, EC-queuing ligand for the EC TIE2 receptor (Fig 1C). Angiopoietin-2 (ANGPT2) is produced by ECs and stored in Weibel-Palade bodies, allowing for rapid release upon EC stimulation. During states of activation or dysfunction, ECs release ANGPT2, which competitively antagonizes ANGPT1 at the TIE2 receptor, thereby promoting barrier destabilization and potentially amplifying the effects of cytokine signaling. Both ANGPT1 and ANGPT2 have been measured in multiple disease states and are discussed separately in the following sections.

**Endothelial Dysfunction and Inflammation**

ECs act as targets and amplifiers of the cytokine signaling associated with particular inflammatory states (eg, post-CBP) or critical illness (eg, sepsis, ARDS, multiple-organ dysfunction).
**Summary of Studies Investigating EC-Derived Markers of Activation or Damage in Children With Sepsis**

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Major Findings</th>
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<tr>
<td>Giuliano et al 53</td>
<td>Observational study of 45 children ranging from healthy control subjects to patients with septic shock</td>
<td>Admission and peak levels of ANGPT2 and ANGPT2/ANGPT1 ratios were elevated in children with severe sepsis and septic shock compared with children with SIRS or control children</td>
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<tr>
<td>Wang et al 52</td>
<td>Observational study of 45 children ranging from healthy control subjects to patients with septic shock</td>
<td>ANGPT1, ANGPT2, and serum bicarbonate demonstrated the strongest correlation with sepsis severity</td>
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<tr>
<td>Wong et al 53</td>
<td>Temporal extension of sepsis biomarkers from 225 children with septic shock</td>
<td>Five biomarkers previously identified, including CCL3, heat shock protein-A1B, IL-8, elastase-2, and lipocalin-2, of which CCL3 and IL-8 correlated with disease severity over time</td>
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<tr>
<td>van der Flier et al 54</td>
<td>Observational study of skin biopsy samples from 10 children with and 28 without meningococcal sepsis</td>
<td>Expression and soluble levels of VEGF-receptor 2 were decreased in children with sepsis and levels inversely correlated with PRISMM score</td>
</tr>
<tr>
<td>Wong et al 55</td>
<td>Derivation of sepsis biomarkers from 220 children with septic shock using regression tree analysis</td>
<td>Five biomarkers were identified, including CCL3, heat shock protein-A1B, IL-8, elastase-2, and lipocalin-2; CCL3 and IL-8 are at least partially of endothelial origin</td>
</tr>
<tr>
<td>Giuliano et al 56</td>
<td>Observational study of 116 children ranging from healthy control subjects to patients with septic shock</td>
<td>ANGPT2 levels were elevated in septic shock and correlated with the category of shock. ANGPT1 levels were decreased in patients with septic shock compared with the other groups</td>
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<td>Pickkers et al 57</td>
<td>Observational study of 13 children with meningococcal sepsis</td>
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<td>Oragui et al 58</td>
<td>Observational study of 18 children with meningococcal sepsis</td>
<td>Urinary excretion of glycocalyx and basement membrane components correlated with degree of capillary leak and proteinuria</td>
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<tr>
<td>Hazeltz et al 59</td>
<td>Observational study of 52 children with meningococcal sepsis</td>
<td>IL-6, IL-8, C5b, C5c, and C5-CRP levels were all significantly different between survivors and nonsurvivors. Complement levels correlated with illness severity score and degree of capillary leak</td>
</tr>
</tbody>
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CCL3, C-C chemokine ligand 3; IL, interleukin; PRISM III, Pediatric Risk of Mortality III; SIRS, systemic inflammatory response syndrome; VEGF, vascular endothelial growth factor.

dysfunction syndrome [MODS], cerebral edema). Upon activation by pro-inflammatory cytokines such as interleukin-1 and tumor necrosis factor, venular ECs synthesize and display adhesion molecules (E-selectin, ICAM-1, and VCAM-1) on their luminal surfaces, capturing circulating leukocytes and chemokines, stimulating leukocyte motility. In settings of local inflammation, these changes are typically limited to ECs lining these postcapillary venules, but in overwhelming systemic processes such as sepsis, this activation may extend to all vascular segments. In addition, ECs produce a variety of cytokines that modulate the inflammatory response, although this function has been incompletely characterized.

Many studies in various disease states have assessed EC-derived serum markers of activation or inflammation in critically ill children. These studies demonstrate an association between these markers and disease severity, or a correlation to a variety of adverse clinical outcomes. However, these markers lack segmental, spatial, and mechanistic specificity. Despite these limitations, such serum markers remain the most frequently used measures of EC function and dysfunction in various inflammatory states.

**Sepsis**

The pathologic inflammatory response to sepsis is largely mediated by the endothelium. Amplified cytokine signaling and nonspecific immune cell activation are hallmarks of early stages of this disease. At later times, counterregulatory mechanisms may impair these processes, interfering with resolution of the underlying infection. Pediatric studies have focused on EC-secreted molecules that intertwine permeability and immune cell recruitment such as ANGPT1, ANGPT2, and soluble adhesion molecules (summarized in Table 2). Taken together, these studies indicate that ECs play a significant role when either activated or damaged during sepsis but fail to implicate specific processes; they highlight the urgent need to develop more definitive assays of vascular function in this setting.

**CPB**

Required for the surgical repair of many congenital heart disease lesions, CPB is widely believed to activate or damage ECs. The clinical manifestation of this response is termed low cardiac output syndrome, and it typically occurs within 8 hours of exposure to the CPB circuit. Potential mechanisms include cytokine release when circulating immune cells are exposed to the artificial CPB circuit, ischemia-reperfusion injury, altered flow dynamics while on CPB, hypothermia and re-warming, or response to blood products.

Various markers of complement, inflammation, and EC activation are cleaved during CPB. Levels of soluble EC adhesion molecules post-CPB have been associated with nonspecific neutrophil activation and impaired regulation of inflammation. Collectively, these studies show decreased levels of all
soluble adhesion molecules during CPB, with lower levels associated with postoperative complications. Higher levels of complement (C3d) are found in patients undergoing CPB compared with those who do not require bypass. Levels of ANGPT2 that are elevated 6 hours after CPB correlate with PICU length of stay.70 Finally, pro-inflammatory cytokines and chemokines (namely, interleukin-6 and interleukin-8, respectively) are also increased after CPB, although the significance remains uncertain.71

**ARDS**

Pulmonary ECs are essential for lung function and modulate vascular tone, locally match perfusion to ventilation, and maintain alveolar integrity.72 The interaction between the alveolar epithelium and pulmonary ECs is disrupted in ARDS and is believed to correlate with clinical severity scores.73 EC may become injured by stimuli originating in the alveolus, so called direct-ARDS, or the bloodstream, termed indirect-ARDS. Disruptions of EC function have been observed in ARDS, and a prevailing theory is that pulmonary microvascular thrombi contribute to dead space.74 Although data are limited in children, 3 studies have examined markers of EC dysfunction in pediatric ARDS. Pediatric patients with ARDS who died developed a more than fivefold increase in plasma levels of soluble intercellular adhesion molecule 1 (sICAM-1); however, although sICAM-1 is elevated on activated ECs, it is also elevated in other cell types.75 Levels of vWF are elevated in children with ARDS and correlate with mortality and length of mechanical ventilation.76 Finally, soluble thrombomodulin had decreased activity in children with ARDS and organ dysfunction.77 This finding is ambiguous because, as noted earlier, the shedding of thrombomodulin may measure EC injury, but thrombomodulin is actually decreased with persistent inflammatory stimulation. Although these studies, taken together, do not conclusively show a causal role of EC injury in ARDS, they do associate endothelial injury with morbidity and mortality.

**MODS**

MODS is believed to represent a common pathway to mortality in the most severely ill children. The pathophysiology of MODS is heterogeneous and complex, involving endothelial, epithelial, and immune cell dysfunction and failure.78 Although the etiology for progression from systemic disease to MODS is usually unknown, the distribution of ECs throughout every organ system suggests an active role.79 In intubated children, levels of VCAM-1 are significantly higher when ≥3 organ systems fail, and levels of E-selectin are higher in patients with infectious etiologies of MODS but do not correlate with the number of failing systems.80 This finding may be explained by the EC response to activation: VCAM-1 expression is sustained while E-selectin transiently upregulated. These studies illustrate the importance of the time-resolved response of ECs to critical illness.

**Cerebral Edema and Traumatic Brain Injury**

ECs establish and maintain the blood–brain barrier via formation of specialized tight junctions with the support of surrounding pericytes and astrocytes. Inherited and somatic mutations of proteins that contribute to interactions among these 3 cell types may lead to cerebral cavernous malformations or stroke.81 Acquired and transient breakdown of these barriers, involving changes in the assembly in multiple different intercellular adherens and tight junctional proteins, are suspected to have a central role in the cerebral edema that accompanies many central nervous system insults. In children, morbidity from traumatic brain injury results from the combination of primary and secondary injuries, and ECs may play important roles in both processes.82 Brain vessel biopsy samples from adults and children with tumors demonstrate disruption of tight junctions inversely correlated with the degree of edema, implicating EC junctional remodeling as a component of cerebral edema.83 In pediatric patients after traumatic brain injury, cerebrospinal fluid levels of ET-1 rose threefold compared with control subjects, although levels were not correlated with cerebral perfusion pressures. In addition, serum ET-1 levels negatively correlated with Glasgow coma scale scores and functional outcomes.84 This study indicates that blood flow is coupled to endothelial function in traumatic brain injury, and more research is needed to elucidate the underlying mechanisms.

**CONCLUSIONS**

During homeostasis, the endothelium is actively involved in the regulation of blood flow, vessel permeability, coagulation, and immune cell activation. The stress of critical illness induces ECs to acquire new functions to restore homeostasis (EC activation). Inappropriately severe or persistent activation may manifest as maladaptive functions (EC dysfunction). This spectrum, from activation to dysfunction, is complex and difficult to assess in critically ill children. Current investigations have relied on indirect, nonspecific biomarkers of EC activation and/or injury. These biomarkers do not provide mechanistic, spatial, or temporal information that is necessary to understand the multifactorial EC response to critical illness. Additional research is needed to identify new diagnostic and therapeutic avenues in critically ill children.
ABBREVIATIONS

ANGPT1: angiopoietin-1
ANGPT2: angiopoietin-2
aPC: activated protein C
ARDS: acute respiratory distress syndrome
ATII: angiotensin-II
CPB: cardiopulmonary bypass
CRT: capillary refill time
EC: endothelial cell
ET-1: endothelin-1
ICAM-1: intercellular adhesion molecule 1
ICAM-2: soluble intercellular adhesion molecule 1
ICAM-3: intercellular adhesion molecule 3
ICAM-5: intercellular adhesion molecule 5
ICAM-6: intercellular adhesion molecule 6
MODS: multiple-organ dysfunction syndrome
NO: nitric oxide
sICAM-1: soluble intercellular adhesion molecule 1
SMC: smooth muscle cell
VCAM-1: vascular cell adhesion molecule 1
vWF: von Willebrand factor

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