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Biomarkers of endothelial activation/dysfunction in infectious diseases

Andrea V Page[1,2,*] and W Conrad Liles[1,2,3]

[1]Division of Infectious Diseases; Sandra A. Rotman Laboratory for Global Health; Mount Sinai Hospital-University Health Network; Toronto, ON Canada; [2]Department of Medicine; University of Toronto; Toronto, ON Canada; [3]Division of Allergy and Infectious Diseases; Department of Medicine; University of Washington School of Medicine; Seattle, WA USA

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Endothelial dysfunction contributes to the pathogenesis of a variety of potentially serious infectious diseases and syndromes, including sepsis and septic shock, hemolytic-uremic syndrome, severe malaria, and dengue hemorrhagic fever. Because endothelial activation often precedes overt endothelial dysfunction, biomarkers of the activated endothelium in serum and/or plasma may be detectable before classically recognized markers of disease, and therefore, may be clinically useful as biomarkers of disease severity or prognosis in systemic infectious diseases. In this review, the current status of mediators of endothelial cell function (angiopoietins-1 and -2), components of the coagulation pathway (von Willebrand Factor, ADAMTS13, and thrombomodulin), soluble cell-surface adhesion molecules (soluble E-selectin, sICAM-1, and sVCAM-1), and regulators of vascular tone and permeability (VEGF and sFlt-1) as biomarkers in severe infectious diseases is discussed in the context of sepsis, *E. coli* O157:H7 infection, malaria, and dengue virus infection.

Introduction

The vascular endothelium is a complex organ that can interact with and respond to its environment, shifting from quiescent to activated and back again. The activated endothelial cell phenotype is permeable, prothrombotic, and pro-inflammatory, and, when localized to a site of infection or injury, is critical for vascular remodeling and repair, leukocyte trafficking, and the sequestration or eradication of pathogens or toxins. However, the same endothelial response can be detrimental if it is uncontrolled, persistent, or widespread. Many common and serious infectious diseases and syndromes, including sepsis, hemolytic-uremic syndrome (HUS), severe malaria, and dengue hemorrhagic fever, are characterized by excessive vascular permeability, microvascular thrombosis, and inflammation that results from diffuse endothelial cell dysfunction.[1-4] In each syndrome, the degree of endothelial activation and subsequent dysfunction contributes to the severity of illness and progression of disease.

*Correspondence to: Andrea V Page; Email: APage@mtsinai.on.ca
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Rapid diagnosis and early intervention can potentially improve clinical outcomes in individuals affected by potentially life-threatening infectious diseases. For patients who present with severe disease, the need for intensive therapy is typically evident from basic clinical and laboratory criteria. However, patients can present at any point along the spectrum of disease severity. Even those who present with mild forms of potentially life-threatening infections may be harboring sub-clinical endothelial cell activation that will ultimately drive progression to severe disease. Peripheral blood biomarkers of endothelial cell activation/dysfunction may therefore be clinically useful to identify those individuals who, from among all patients with a particular infection or infectious syndrome, will progress to severe disease. Examples include patients with bacteremia who subsequently develop sepsis, individuals with *E. coli* O157:H7 diarrheal disease that subsequently progress to HUS, individuals with malaria who subsequently develop severe or cerebral malaria, and individuals with dengue virus infection who subsequently progress to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Moreover, the intensity of endothelial activation/dysfunction may yield prognostic information even among already critically ill patients, and markers of endothelial activation/dysfunction have been investigated in this context as well.

In this review, we will examine the biology and clinical utility of a variety of proposed biomarkers of endothelial activation/dysfunction that have been studied for either diagnosis or prognosis in one or more infectious diseases (see **Table 1** for a list of biomarkers, associated infectious diseases, and cited references). Because of the breadth and depth of the literature in this field, the review will focus primarily on clinical studies, with reference to in vitro or animal models when necessary to provide supporting evidence or rationale, and will include only those proposed biomarkers that have been studied in multiple infectious diseases or syndromes. Furthermore, the review will address only molecules that reflect endothelial activation, and will not address those that precipitate endothelial activation. Ultimately, any candidate biomarker, whether used for diagnosis or prognosis, should be judged against certain universal criteria proposed for a hypothetical ideal biomarker.[5] Adapted for the endothelium, a valid biomarker should be specific for endothelial activation/dysfunction, indicative of the underlying pathophysiology of the disease, reproducible across patients and populations, useful in

Table 1. Summary of biomarkers and cited references, by disease

Biomarker	References
Mediators of endothelial function	
Angiopoietin-1	
Sepsis	22 and 23
HUS	26
Malaria	28–33
Dengue	34
Angiopoietin-2	
Sepsis	7–16, 18–21, and 25
HUS	26
Malaria	27–33
Dengue	34
Components of the coagulation pathway	
von Willebrand Factor	
Sepsis	16 and 37–39
HUS	N/A
Malaria	30, 40, and 41
Dengue	42
ADAMTS13	
Sepsis	37, 39, 46, and 47
HUS	N/A
Malaria	48 and 49
Dengue	42
Thrombomodulin	
Sepsis	51–54
HUS	N/A
Malaria	55–57
Dengue	58 and 59
Soluble cell-surface adhesion molecules	
sE-selectin, sICAM-1, sVCAM-1	
Sepsis	61–79
HUS	80 and 81
Malaria	30, 41, and 82–85
Dengue	86 and 87
Mediators of vasomotor tone and permeability	
VEGF	
Sepsis	8, 23, and 89–93
HUS	95
Malaria	27 and 96
Dengue	97–100
sFlt-1	
Sepsis	90, 91, and 94
HUS	N/A
Malaria	41 and 96
Dengue	97

sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

a single measure, and correlated with disease activity over time, and should add information to clinical judgment.

Mediators of Endothelial Function: Angiopoietin-1 and -2

Angiopoietin-1 and -2 (Ang-1 and Ang-2) are two of the most widely studied biomarkers of endothelial activation/dysfunction in infectious diseases. As reviewed by van Meurs et al., Ang-1 is produced constitutively, primarily in the pericytes and smooth muscle cells that surround the endothelial cell monolayer.[6] Ang-2 is produced by endothelial cells themselves and stored in Weibel–Palade bodies for rapid release upon exposure to various noxious or inflammatory stimuli. Ang-1 and Ang-2 are antagonistic ligands of the Tie-2 receptor, which belongs to a family of vascular tyrosine kinase receptors expressed primarily in endothelial cells. Under normal circumstances, the serum concentration of Ang-1 exceeds that of Ang-2, allowing Ang-1 to preferentially bind the Tie-2 receptor and thereby initiate pro-survival pathways and inhibit pro-inflammatory pathways. The net result is endothelial cell quiescence. In contrast, inflammation stimulates Weibel–Palade body exocytosis and Ang-2 release, allowing Ang-2 to preferentially bind the Tie-2 receptor and promote pro-inflammatory and pro-thrombotic pathways, as well as microvascular leak. Angiopoietin dysregulation indicates a perturbation of the normally low Ang-2:Ang-1 ratio, whether by decreased Ang-1, increased Ang-2, or both.[7]

Prospective observational studies in sepsis have generated the bulk of the evidence in support of the angiopoietins as both diagnostic and prognostic biomarkers. In general, although most studies were small and the differences did not always reach statistical significance, patients with sepsis have higher plasma/serum levels of Ang-2 compared with healthy adults, non-septic hospitalized patients, and patients with non-infectious systemic inflammatory response syndrome (SIRS) or critical illness.[8-13] A number of groups have also reported that the magnitude of Ang-2 dysregulation correlated with the severity of sepsis, as defined by either the Sequential Organ Failure Assessment (SOFA) score or the Acute Physiology And Chronic Health Evaluation (APACHE) II score.[8,10,12-14]

Elevated levels of Ang-2 have also been linked to mortality in sepsis. A small study of 21 septic patients found higher Ang-2 levels, both on admission and at 72 h, in non-survivors than survivors at day 28.[15] Likewise, in a study of 50 critically ill patients, plasma Ang-2 concentrations measured within 12 h of the diagnosis of sepsis were predictive of ICU and 28-d mortality.[16] vWF was not as uniformly associated with illness severity in this study, prompting the authors to speculate that, despite co-storage in Weibel–Palade bodies, differential regulation might be relevant.[17] Other small studies documented the same relationship between Ang-2 concentration at or near the time of clinical recognition of sepsis and eventual mortality.[10,13] Likewise, Ang-2 concentrations have been reported to be predictive of mortality in certain subsets of critically ill patients, including those requiring renal replacement therapy and in children with septic shock.[18,19] Importantly, plasma Ang-2 levels

obtained from individuals with suspected infection within one hour of presentation to the emergency department were shown to be proportional to disease severity and were predictive of the development of septic shock within 72 h and of overall mortality.[20] For each outcome in this study, Ang-2 levels at baseline were more predictive than those of either soluble intercellular adhesion molecule-1 (sICAM-1) or soluble E-selectin, both also markers of endothelial activation. Cut-off values for Ang-2 derived from this cohort performed well in a validation cohort of 85 patients: an Ang-2 cut-off value of 2.86 ng/mL predicted the development of severe sepsis with a sensitivity of 75% and specificity of 56%, an Ang-2 cut-off value of 3.56 ng/mL predicted the development of septic shock with a sensitivity of 73% and specificity of 68%, and an Ang-2 cut-off value of 5.1 ng/mL predicted mortality with a sensitivity of 80% and specificity of 76%. This study by David et al. is unique in that it approximated how Ang-2 levels might be used as biomarkers in clinical practice—i.e., levels would be measured early in disease and used to inform clinical decision-making regarding prognosis on the basis of well-defined cut-off values.

However, it should be noted that not all studies have demonstrated a similar relationship between Ang-2 concentration and mortality in sepsis. Parikh et al. found a correlation between serum Ang-2 levels and impaired pulmonary gas exchange (a PaO2/FiO2 ratio of <200) in 22 critically ill patients with sepsis, but not with survival or APACHE II score.[9] Davis et al. documented an association between Ang-2 and both the APACHE II and SOFA scores, but not with mortality in their study of 85 hospitalized patients with sepsis.[8] Importantly, overall mortality in this study was unusually low. In a recent observational study by Calfee et al., Ang-2 and vWF were measured in 931 patients with acute lung injury (ALI).[21] Median plasma levels of both Ang-2 and vWF taken on the day of enrollment were highest in patients with sepsis-induced ALI than in ALI from any other cause, and both were higher in non-survivors at 90 d than in survivors. Ang-2 and vWF were independently predictive of survival at 90 d even in a combined model. Interestingly, Ang-2 was a stronger predictor of mortality in patients with a non-infectious cause of ALI than in patients with infection-induced ALI, in whom Ang-2 was more uniformly elevated in both survivors and non-survivors. In a multivariate analysis of patients with infection-induced ALI, baseline Ang-2 levels were no longer predictive of mortality. No similar relationship to the cause of ALI was found with vWF.

Taken together, the variable results from these studies demonstrate that multiple factors influence both Ang-2 levels and mortality in critical illness and that eventual clinical use of the angiopoietins, or any other biomarker, in sepsis will be predicated on the definition of a very specific patient population in whom the test is applicable.

In contrast to the wealth of studies that have investigated Ang-2 as a biomarker in sepsis, fewer have focused on Ang-1 for the same indication. Median serum Ang-1 concentrations are lower in critically ill patients with or without sepsis than in healthy controls.[15] At ICU admission, the plasma Ang-1 concentration predicted 28-d mortality in 70 patients with severe sepsis, while decreased Ang-1 levels were also associated with mortality in a study of Malawian children with severe bacterial infection.[22,23] On the other hand, plasma Ang-1 concentrations did not differ between survivors and non-survivors in a study of 50 critically ill patients with sepsis.[16] It is possible that the clinical utility of Ang-1 as a prognostic biomarker may be limited by the frequent fluctuations in circulating levels seen in individual patients with hourly sampling over a 24-h time period.[7] Finally, the Ang-2:Ang-1 ratio, reflecting perturbations in one or both of the angiopoietins, has been found to be predictive of the eventual development of septic shock at the time of fever onset in neutropenic patients.[24]

The angiopoietins have also been studied, though much less extensively, in other infectious diseases. In invasive group A β-hemolytic streptococcal disease, the Ang-2:Ang-1 ratio was higher in patients with streptococcal toxic shock syndrome (STSS) than in those with invasive infection without shock.[25] Both the absolute Ang-2 concentration and the Ang-2:Ang-1 ratio effectively discriminated between infected patients with and without STSS, making the angiopoietins possible diagnostic biomarkers in this infection. As mortality in patients with STSS was numerically, but not statistically, different from that in patients with invasive infection without shock, no correlation between angiopoietin dysregulation and mortality was found.

Angiopoietin dysregulation has also been associated with disease severity in *E. coli* O157:H7 infection, although in this infection dysregulation was primarily manifested as decreased Ang-1. The median serum Ang-1 concentration was lowest in patients with HUS, but was also reduced in children with *E. coli* O157:H7 infection who eventually developed HUS but had yet to do so.[26] Furthermore, serum Ang-1 levels could partially discriminate between two clinically indistinguishable groups: patients with uncomplicated infection and those who would eventually develop HUS. A serum Ang-1 cut-off value of 61 000 pg/mL could predict the eventual development of HUS with a sensitivity of 70% and a specificity of 69%. Notably, this pre-HUS group had normal renal function and platelet counts, indicating that angiopoietin dysregulation was independent of these potentially confounding factors.

In *Plasmodium falciparum* malaria, plasma Ang-2 levels were found to be higher in patients with severe vs. non-severe disease, and were a better predictor of outcome than venous lactate.[27] Subsequent studies, however, have all identified Ang-1 as a more consistent diagnostic biomarker in malaria, discriminating between cerebral malaria and uncomplicated malaria in Thai adults and Ugandan children, between cerebral, severe, and uncomplicated malaria in Thai adults, and between cerebral malaria with retinopathy and febrile, non-malarial causes of decreased level of consciousness in Malawian children.[28-30] Furthermore, decreased Ang-1 was associated with *P. falciparum* malaria in pregnancy, while an increased Ang-2:Ang-1 ratio was associated with both placental malaria and low birth weight infants.[31] As a prognostic marker, the plasma Ang-2 concentration at admission to hospital has been shown to correlate with mortality in children with cerebral malaria in two separate studies.[32,33]

Finally, a small study of 49 Indonesian children with dengue virus infection suggests that angiopoietin dysregulation

(decreased plasma Ang-1 and increased plasma Ang-2) is present in both dengue hemorrhagic fever and dengue shock syndrome, and resolves with convalescence, indicating that the angiopoietins may have a role as diagnostic biomarkers in this infection as well.[34] To date, no studies have attempted to correlate angiopoietin dysregulation with outcome in dengue virus infection or dengue hemorrhagic shock.

Components of the Coagulation Pathway: von Willebrand Factor, ADAMTS13, and Thrombomodulin

Endothelial cells play a key role in maintaining blood flow and preventing coagulation in the absence of vascular injury. Quiescent endothelial cells generate activated protein C on the cell surface, produce tissue-type plasminogen activator (tPA) to stimulate fibrinolysis, and inhibit thrombin formation and platelet adhesion, among other anti-thrombotic actions.[35] In the presence of activating stimuli, however, the endothelial cell phenotype becomes pro-coagulant, and the expression and secretion of anticoagulant factors is reduced. Since infectious pathogens are important catalysts for endothelial activation, levels of endothelial-derived soluble, cleaved membrane-bound, or secreted components of the coagulation pathway have been investigated as diagnostic or prognostic markers of endothelial activation/dysfunction in infectious diseases. As with Ang-1/2, endothelial-associated coagulation pathway biomarkers have been most frequently studied in sepsis, and therefore, their use in this context is the focus of the following discussion.

von Willebrand Factor (vWF) acts to stabilize the adhesion of platelets at sites of vascular injury. It is stored in the Weibel–Palade bodies of endothelial cells for release upon endothelial cell activation.[36] Despite co-localization, however, vWF does not always perform similarly to Ang-2 as a biomarker, and therefore, warrants independent study and analysis.[16,21] The role of vWF as a diagnostic biomarker has been examined in two small studies of sepsis, with each enrolling fewer than 20 patients. Nonetheless, both studies reported that plasma vWF levels were elevated in critically ill patients with sepsis as compared with healthy adults, patients with SIRS, and non-septic hospitalized patients.[37,38] The study by Claus et al. also investigated the role of vWF as a prognostic marker, and found an association between vWF level at the time of enrollment and eventual mortality. Similarly, a study of 50 mechanically ventilated patients in the ICU, enrolled within 12 h of the diagnosis of sepsis, found that the vWF concentration on the day of enrollment, but not thereafter, was predictive of mortality during ICU admission.[16] However, not all studies have confirmed the association between vWF levels and mortality in sepsis.[39]

vWF has shown more promise as a marker of endothelial activation/dysfunction in severe and/or cerebral malaria. Elevated levels of vWF have been reported consistently in both adults and children with malaria, and have been associated with disease severity in all studies published to date that have attempted to address the issue.[30,40,41] vWF levels are higher in malaria than in non-malarial febrile illness, higher in cerebral malaria or severe malarial anemia than in uncomplicated malaria, and higher in cerebral malaria with retinopathy than in non-malarial illness with fever and altered level of consciousness. vWF levels also decline with convalescence, but have not been shown to differentiate between survivors vs. non-survivors in severe malaria.[40,41] Nonetheless, the ability to discriminate between malaria and non-malarial febrile illnesses, as documented in two studies, suggests a higher degree of specificity for vWF levels in malaria than in sepsis, and suggests that vWF deserves consideration as a marker of severe or cerebral malaria.

Finally, vWF has also been explored, in a single study, as a biomarker in dengue virus infection. In Indonesian children with dengue (43 with dengue hemorrhagic fever [grade I and II] and 30 with dengue shock syndrome [grade III and IV]), vWF was not consistently correlated with disease severity.[42] The median plasma concentration of vWF at enrollment was highest in children with dengue hemorrhagic fever (DHF), followed by those with dengue shock syndrome (DSS); both groups, however, demonstrated median plasma vWF levels higher than those found in uninfected children. Notably, significant overlap between vWF levels in individual patients in all three groups would preclude the use of vWF as the sole diagnostic biomarker to differentiate DHF from DSS. Furthermore, vWF levels at enrollment did not predict eventual mortality even in the most severely ill children.

Also produced in endothelial cells and closely connected with vWF in the process of coagulation is ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type-1 motif, number 13), which differs from vWF in that it is released constitutively without prior storage.[43] Once released, ADAMTS13 cleaves biologically active and prothrombotic ultra-large vWF multimers and inhibits thrombus formation.[44] ADAMTS13 has been shown, in animal models, to decline during systemic infection. For instance, in the well-characterized murine cecal ligation and puncture (CLP) model of polymicrobial sepsis, ADAMTS13 is significantly reduced.[45] Because the effect of CLP on ADAMTS13 levels is abrogated in $vWF^{-/-}$ (knockout) mice, the authors postulated that low ADAMTS13 levels in sepsis likely reflect consumption of the protease in the face of high plasma concentrations of vWF resulting from Weibel–Palade body exocytosis following endothelial activation. Despite promising findings in animal models, studies in human subjects have yielded decidedly mixed results. In a study of 58 children with severe meningococcal sepsis, decreased levels of ADAMTS13 were observed, correlating with disease severity based on the Pediatric Risk of Mortality score.[46] Levels were also lower in non-survivors as compared with survivors. In a study by Martin et al., median ADAMTS13 levels were highest in healthy adults, reduced in adults with non-infectious causes of organ failure, and lowest in patients with severe sepsis, in whom ADAMTS13 deficiency was associated with mortality.[47] As expected, a reciprocal relationship with plasma vWF levels was found, with the highest vWF concentrations occurring in patients with severe sepsis. Notably, however, marked overlap in ADAMTS13 values was present between individual patients in all three groups. Furthermore, studies in similar populations have failed to confirm these results. In a study of 40 adult patients with severe sepsis or septic shock,

ADAMTS13 activity was lower in patients than healthy controls, but was not correlated with organ dysfunction at study entry nor ultimately with mortality.[39] It should be noted that neither increased vWF nor decreased ADAMTS13 is specific for sepsis, as both are dysregulated in inflammation regardless of the presence of an infectious precipitant.[37]

Low levels of ADAMTS13 have also been documented in dengue hemorrhagic fever and dengue shock syndrome, notably in the study by Djamiatun et al.; however, ADAMTS13 levels at enrollment were insufficient to predict mortality in this cohort.[42] The authors speculated that endothelial activation caused by dengue virus-induced pro-inflammatory cytokines, followed by Weibel–Palade body exocytosis, vWF release, and subsequent ADAMTS13 consumption may have accounted for their findings. A similar hypothesis could be generated to account for the ADAMTS13 deficiency and ultra-large vWF multimer excess found in two studies, one of patients with severe and cerebral *P. falciparum* malaria and the other of patients with milder infection, categorized only as symptomatic *P. falciparum* or *Plasmodium vivax* malaria.[48,49] To date, no published studies have correlated ADAMTS13 deficiency with outcome in malaria.

In contrast to the secreted molecules vWF and ADAMTS13, thrombomodulin (TM) is present in large quantities on the surface of the endothelium, particularly in the microcirculation, where it acts as an anticoagulant. The TM-thrombin complex catalyzes the generation of the anticoagulant molecule activated protein C, and prevents thrombin from converting fibrinogen to fibrin and from exerting other procoagulant effects.[50] Consistent with the procoagulant properties of the activated endothelium, cell-surface thrombomodulin expression is reduced during sepsis, likely secondary to shedding of the molecule.[51] Soluble TM (*s*TM) has therefore been proposed as both a diagnostic and prognostic marker of endothelial activation/dysfunction. In a study of 37 patients with blunt trauma, those with a *s*TM level above the upper limit of normal within 6 h post-trauma were more likely to develop sepsis or multi-organ dysfunction than patients whose *s*TM level was within the normal range.[52] In meningococcal sepsis, *s*TM correlated with disease severity.[51] In a substudy of the PROWESS recombinant activated protein C trial in patients with severe sepsis, *s*TM also correlated with APACHE II score as a marker of disease severity.[53] Although levels of *s*TM were higher in non-survivors than survivors, large inter-individual variability and overlap between the groups likely precludes the use of *s*TM as the sole prognostic marker in sepsis. Similarly, in a study of 22 children with septic shock, non-survivors had significantly higher mean plasma *s*TM levels measured over the first 72 h after diagnosis than did survivors.[54]

In malaria, *s*TM levels are likewise elevated.[55] In a study of 555 Malian children with uncomplicated and non-cerebral severe malaria, *s*TM levels increased with infection and declined with convalescence.[56] Moreover, levels of *s*TM were shown to correlate with both parasitemia and disease severity, and were higher in children with severe malaria than in those with uncomplicated malaria. In a small study of patients with uncomplicated *P. falciparum* malaria, *s*TM was higher in patients vs. uninfected control subjects, and was positively correlated with levels of pro-inflammatory cytokines and anemia, a marker of disease severity.[57]

Two studies have correlated *s*TM with disease severity in dengue hemorrhagic fever, in which vascular leak is a prominent manifestation of disease. A study of 111 Thai children with dengue fever, dengue hemorrhagic fever, or other febrile illness reported higher levels of *s*TM in the subset of patients with dengue shock syndrome, while a study of 48 children in Vietnam demonstrated that *s*TM levels increased commensurate with the severity of the dengue shock syndrome.[58,59] In the latter study, however, there was significant overlap between *s*TM values in those with mild DSS vs. those with moderate/severe DSS, again limiting the clinical use of *s*TM as the sole marker of disease severity in dengue virus infection.

Endothelial Cell-Surface Adhesion Molecules: sE-Selectin, sICAM-1, and sVCAM-1

Differential cell surface molecule expression between quiescent and activated endothelial cells influences not only the relative balance between pro- and anti-coagulant activity, but also the degree of adhesion of circulating blood cells. E-selectin is expressed on activated endothelial cells, where, in combination with P-selectin, it facilitates rolling of leukocytes along the endothelial layer as a prelude to leukocyte adhesion (facilitated by the upregulation of ICAM-1 and VCAM-1 [vascular cell adhesion molecule-1]) to activated endothelial cells and subsequent transmigration across the endothelial barrier to a site of injury or inflammation.[60] Given their specificity for endothelial cells in the activated state, it is not surprising that soluble forms of these cell-surface molecules, shed from endothelial cells after activation, have been widely studied, often together, as diagnostic and prognostic markers in a variety of infectious diseases.

In sepsis, sE-selectin, sICAM-1, and sVCAM-1 have all been studied in relation to disease severity and outcome. However, study results have been inconsistent and occasionally contradictory, perhaps because the utility of the soluble cell-surface adhesion molecules as biomarkers in sepsis is dependent on both the population under study and the precise point in illness at which each is measured. Serum concentrations of both sE-selectin and sICAM-1 are elevated in septic neonates as compared with their hospitalized, non-septic counterparts, with sICAM-1 slightly outperforming sE-selectin as a diagnostic biomarker based on areas under the ROC curves of 0.79 and 0.72, respectively.[61] A serum sICAM-1 cut-off value of 228 ng/mL had a sensitivity of 76.7% and a specificity of 75.6% for the diagnosis of neonatal sepsis, while sE-selectin predicted mortality in a similar study of septic neonates.[62] In children, plasma sE-selectin, sICAM-1, and sVCAM-1 concentrations are higher in those with sepsis on day 1 of diagnosis when compared with healthy controls or acutely ill non-septic children.[63,64] Both sICAM-1 and sVCAM-1, but not sE-selectin, predicted mortality in this population.

In adults, a recent study examined baseline levels of sE-selectin, sICAM-1, and sVCAM-1 in 162 matched patient pairs selected from a larger prospectively enrolled cohort of community-dwelling individuals.[65] In a univariate analysis, patients

who would later develop sepsis had significantly higher baseline levels of sE-selectin and sVCAM-1 than did matched patients who were hospitalized for serious infection without sepsis. After adjusting for vascular risk factors, however, only sE-selectin and sICAM-1 were associated with eventual sepsis. In adult patients at the time of diagnosis of sepsis, levels of sE-selectin, sICAM-1, and sVCAM-1 have been frequently reported to be higher than corresponding levels in non-septic, critically ill patients or healthy controls.[66-71] Interestingly, one study reported higher plasma levels of sVCAM-1 and lower levels of sE-selectin in patients with candidal, as opposed to bacterial, sepsis, suggesting that biomarker combinations may be diagnostically useful for the microbial etiology of sepsis as well.[72] However, a prospective observational cohort study of 161 patients presenting to the emergency department with hypotension found that only sE-selectin and sFlt-1, but not sICAM-1, sVCAM-1 or VEGF, were significantly elevated in 69 patients with sepsis compared with patients with hypotension of other etiology.[73] The area under the ROC curve was 0.74 for sE-selectin and 0.70 for sFlt-1 for the diagnosis of sepsis among patients with hypotension. This study is one of the few to attempt to control for other confounding variables through multivariate logistic regression analysis, an important consideration given that all five putative markers were significantly different among septic and non-septic hypotensive patients by univariate analysis alone. Likewise, correlation between each of the three potential biomarkers and the severity of disease in sepsis has been variable, with some studies reporting modest associations between each individual biomarker and SOFA or APACHE II scores and others reporting no association.[74,75]

Similar contradictory findings have been reported in studies investigating the relationship between these markers and outcomes in sepsis. In those studies that demonstrated a relationship between sE-selectin levels and mortality in sepsis, the kinetics of the elevated sE-selectin levels varied, with increased concentrations persisting for only 3 or up to 7 d after the diagnosis of sepsis.[68,71] In a study of 25 critically ill patients, the serum level of sICAM-1, but not sE-selectin, at the time of diagnosis of sepsis was an independent predictor of mortality in a logistic regression analysis, while in a study of 64 critically ill patients with and without meningitis, sICAM-1 was again the sole predictor of mortality, with neither sE-selectin nor sVCAM-1 associated with outcome.[68,76] In the former, an sICAM-1 cut-off value of 715 ng/mL predicted mortality with a sensitivity of 90% and specificity of 80%, and similar results were obtained in a very small study of 14 patients with septic shock in whom an sICAM-1 value of 800 ng/mL within 24 h of the diagnosis of septic shock was predictive of mortality with a sensitivity and specificity of 71.4%.[77] However, other studies have demonstrated exactly the opposite relationship. In a study of 92 patients with SIRS (infectious or non-infectious), sE-selectin and sVCAM-1, but not sICAM-1, were associated with 28-d mortality in a multivariate analysis.[78] As with sE-selectin, kinetics may also influence the prognostic utility of sICAM-1 and sVCAM-1. In a study of 147 patients including 101 individuals with severe sepsis, serum concentrations of sICAM-1, but not sVCAM-1, were higher in patients with sepsis than in non-septic hospitalized patients at the time of diagnosis.[79] However, only sVCAM-1, and not sICAM-1, was associated with mortality, and its predictive value was only apparent 48 and 120 h after diagnosis. Neither marker was predictive of mortality at baseline.

Two studies have evaluated soluble cell-surface adhesion molecules as biomarkers in HUS. In the first, neither sE-selectin nor sICAM-1 nor sVCAM-1 could differentiate between children with HUS and those with uncomplicated *E. coli* O157:H7 infection. In the second study, only sVCAM-1, but not sE-selectin, was significantly higher among children with diarrhea-associated hemolytic-uremic syndrome than among healthy controls, suggesting that the soluble cell-surface adhesion molecules are not useful as diagnostic biomarkers in this syndrome.[80,81]

Similar inconsistency has been demonstrated in studies of soluble cell-surface adhesion markers in malaria. Patients with severe *P. falciparum* malaria have higher plasma concentrations of sE-selectin, sICAM-1, and sVCAM-1 than patients with mild malaria or mild non-malarial illness, and in one study, plasma levels of sICAM-1 and sVCAM-1, but not sE-selectin, were significantly higher than in patients with sepsis as well.[82,83] However, at least one study has documented higher levels of sE-selectin in patients with non-cerebral severe *P. falciparum* malaria than in those with cerebral malaria, even after adjustment for parasitemia.[84] Likewise surprising, higher plasma concentrations of sE-selectin, sICAM-1, and angiopoietin-2 were reported in Indonesian patients with uncomplicated *P. vivax* malaria when compared with those with uncomplicated *P. falciparum* malaria, despite a lower parasite burden in the former. In Malawian children, sICAM-1 was able to discriminate between uncomplicated and cerebral malaria, but not between cerebral malaria and non-malarial febrile illness with altered level of consciousness.[30] Taken together, these studies suggest that peripheral blood levels of soluble cell-surface adhesion markers are elevated in severe malaria, but that these elevations may not be sufficiently specific to warrant their use as diagnostic biomarkers.

In studies of malaria prognosis, only sE-selectin and sICAM-1 have been associated with outcome. In a relatively large study of 212 Cameroonian children, plasma concentrations of sE-selectin and sICAM-1, but not sVCAM-1, were significantly higher among non-survivors than survivors.[85] In Ugandan children, sICAM-1 levels were higher in non-survivors than in survivors of severe malaria, and, of 12 markers of endothelial activation or inflammation studied, sICAM-1 proved the best predictor of outcome in children with severe malaria, with a statistically significant and clinically respectable area under the ROC curve of 0.84.[41] A plasma cut-off level of 645.3 ng/mL for sICAM-1 yielded a sensitivity of 87% and a specificity of 75% for the prediction of mortality among children with severe malaria.

Finally, the soluble cell-surface adhesion markers have been studied in dengue virus infection, in which sVCAM-1, but not sE-selectin or sICAM-1, was found to be elevated in dengue shock syndrome as compared with acute dengue fever.[86,87] However, the degree of elevation of sVCAM-1 was insufficient to permit differentiation between dengue shock syndrome and dengue hemorrhagic fever.

Mediators of Vascular Tone and Permeability: VEGF and sFlt-1

Vascular endothelial growth factor (VEGF) is a key regulator of normal angiogenesis, during which it promotes endothelial cell survival, growth, and migration.[88] However, it also plays a crucial role in the disruption of the endothelium during inflammation, in which VEGF promotes vasodilatation and permeability. The effects of VEGF are mediated through two receptors, VEGFR1, or the Fms-like tyrosine kinase receptor 1 (Flt-1) and VEGFR2, or the Fms-like tyrosine kinase receptor (Flk-1). Both the transmembrane and soluble form of Flt-1 (sFlt-1) may act to sequester VEGF and prevent binding to Flk-1, which is capable of transducing a much stronger signal. In this way, sFlt-1 may function to fine-tune the actions of VEGF.

While most authors agree that circulating levels of VEGF are elevated in sepsis, consistent with the role of VEGF in inflammation-induced vascular permeability, studies have yielded conflicting results as to whether the degree of elevation correlates with disease severity. In 13 critically ill children with invasive meningococcal disease, plasma VEGF concentrations were highest in those with shock, and correlated with disease severity at presentation as measured by the Pediatric Risk of Mortality Score.[89] In 83 adults with suspected infection, plasma VEGF and sFlt-1 levels upon presentation to the emergency department were higher in those with than without shock, and correlated with the APACHE II (VEGF and sFlt-1) and SOFA (sFlt-1) scores.[90] However, in a study of 83 adult patients with sepsis enrolled within 24 h of admission to the intensive care unit or within 36 h of admission to a hospital ward, there was no significant difference in plasma VEGF levels between those with and without organ dysfunction.[8] In 41 adults with febrile neutropenia, serum VEGF and sFlt-1 concentrations measured 48 h after, but not at the time of, fever onset were significantly higher among those patients who subsequently developed septic shock. The areas under the ROC curves were 0.76 and 0.87 for VEGF and sFlt-1 respectively, for the development of shock.[91]

VEGF has also been studied as a prognostic biomarker in sepsis, with similarly contradictory results. In a small study of 18 critically ill patients, plasma VEGF levels were found to be higher in non-survivors than survivors, while in a larger study of 250 critically ill patients, serum VEGF levels were lower in non-survivors than survivors, both at the time of diagnosis and 72 h later.[92,93] All patients in both studies had severe sepsis. In a large study of 293 Malawian children with severe bacterial infection, plasma VEGF concentrations were higher in survivors than in non-survivors.[23] However, this difference did not remain significant in a multivariate analysis. sFlt-1 has been studied in 81 adult patients with septic shock due to pneumonia, and found to be predictive of 28-d mortality.[94] Plasma levels of sFlt-1, but not VEGF, were significantly higher in non-survivors than survivors. The optimal cut-off point of 224.11 ng/mL for sFlt-1 yielded a sensitivity of only 65% and a specificity of 82% for the prediction of 28-d mortality in patients with pneumonia-related septic shock. The divergent results may reflect the fact that endothelial activation/dysfunction in sepsis depends on the balance or ratio between VEGF, a mediator of endothelial activation, and sFlt-1, a putative mediator of endothelial quiescence.

Studies of VEGF and sFlt-1 as biomarkers in other infectious diseases have yielded similarly inconsistent results. Serum and plasma VEGF levels were reported to be elevated in children with diarrhea-associated HUS as compared with children with renal failure of other etiologies and healthy controls, but no published study has attempted to use VEGF or sFlt-1 to predict the development of HUS during *E. coli* O157:H7 infection.[95] In 146 adult patients in Indonesia with *P. falciparum* malaria, plasma concentrations of VEGF were lower in patients with severe malaria than in healthy controls or those with moderately severe malaria.[27] There was no correlation with mortality. In contrast, elevated plasma levels of sFlt-1 were predictive of mortality in a large study of 156 Ugandan children with malaria.[41] In a study of 465 women in Malawi, plasma concentrations of sFlt-1 were significantly lower in postpartum women with placental malaria than in uninfected controls, while plasma levels of VEGF were not significantly different.[96] However, the discriminatory ability of sFlt-1 was suboptimal: a cut-off level of 16.9 ng/mL had a sensitivity of 52% and a specificity of 84% for the diagnosis of occult placental malaria. Finally, in patients with dengue virus infection, serum sFlt-1, but not VEGF, differentiated between patients with dengue fever and dengue hemorrhagic fever.[97] At a cut-off value of 350 pg/mL, sFlt-1 levels predicted dengue hemorrhagic fever with a sensitivity of 79% and a specificity of 78%. While some studies confirm the lack of association between VEGF and severity of illness in dengue virus infection, others have documented significantly higher VEGF levels in patients with dengue hemorrhagic fever.[98-100]

Conclusion

Multiple candidate biomarkers of endothelial activation/dysfunction have been proposed for use in a variety of infectious diseases. By providing information about the functional status of the endothelium, these molecules act as markers of the extent of the pathophysiologic abnormalities induced by the disease process, and may be more sensitive indicators of worsening disease severity than are traditional laboratory markers alone. However, no biomarker of endothelial activation/dysfunction has yet been shown to provide consistent clinical utility as either a diagnostic or prognostic indicator in sepsis, HUS, malaria, or dengue virus infection. Moreover, no biomarker has achieved the prescribed criteria for the ideal biomarker: although all reflect the endothelial activation/dysfunction that underlies each of these infections, few have been uniformly reproducible across patient populations, clinically useful regardless of time of sampling, and able to identify a subset of patients at high risk for complications or mortality before this risk is evident on the basis of classically recognized markers of each disease. Of the proposed biomarkers of endothelial activation/dysfunction discussed above, Ang-1/2 most closely approximate the ideal. Increased plasma/serum Ang-2 is associated with both disease severity and prognosis in sepsis and has been shown to be predictive of the development of shock and mortality when measured upon presentation to the emergency

department in patients with suspected infection, while decreased Ang-1 in the peripheral blood correlates with disease severity and outcome in *P. falciparum* malaria.

However, even in the field of angiopoietin research there remains the need for further study in the form of rigorous clinical trials. Few candidate molecules have been compared directly in terms of their performance characteristics in well-defined, reproducible, clinically relevant patient populations. Furthermore, there is no consensus as to the best sample (plasma or serum), optimal time of sampling or need for repeated measurements, use of an absolute value or a trend, or ideal cut-off level for any proposed marker. Even for those biomarkers that are reported to differentiate various patient populations in a single infectious disease or syndrome, the degree of overlap between patient groups precludes the use of a single marker for either diagnosis or prognosis. This observation suggests that future studies should focus on combinatorial biomarker strategies for diagnostic and prognostic clinical utility. In addition, no study has yet attempted to link the use of a biomarker to improvements in clinical care or outcome, a crucial step before widespread implementation. Also critical is the development of either high throughput or point-of-care testing. Together, documented clinical impact and an automated testing system are the barriers that must be overcome to translate these potential markers from bench to bedside.

Nevertheless, the field of research into biomarkers of endothelial activation/dysfunction has yielded important insights into the endothelial abnormalities that contribute to the clinical syndromes seen in various infectious diseases and has provided the rationale for studies of novel therapeutics. Endothelial-based therapies could target the final common pathway in the pathogenesis of a variety of infectious diseases, allowing generalized therapy to be initiated while waiting for the identification of a specific pathogen. Such therapies could revolutionize care for patients with suspected infection without clear source in both the emergency department and intensive care unit, and lead to improved clinical outcomes in selected patient populations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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