Trauma is the leading cause of death during the first 4 decades of life and kills more Americans than diseases such as cancer, heart disease, and lung disease combined over this time span. Each year, more than 2 million people encounter traumatic injuries, and of these people, more than 150,000 die as a result of their injuries. Although this toll is shocking, when one considers the lost economic productivity of trauma patients coupled with the expense of caring for them, the numbers become truly staggering and exceed more than $130 billion annually. Classically, death caused by trauma follows a trimodal distribution: immediate, early, and late. The immediate deaths occur in the field and are caused by lethal injuries, such as massive head trauma, or exsanguination from devastating injuries, such as torn aortas or hepatic avulsions. Early trauma deaths occur in the first few hours following injury and are caused by traumatic brain injury and herniation or ongoing hemorrhage. The last group of deaths, the late group, typically occurs in the intensive care unit several days after trauma because of septic complications and multiorgan failure. In this last group of patients, the early detection of infectious complications through the application of suitable biomarkers can have, perhaps, its greatest effect on improved outcomes following severe trauma.

A biomarker can be defined as a clinical indicator that can be directly measured and evaluated as a sign of a physiologic, pathogenic, or pharmacologic response. For the biomarker to have real clinical relevance, it needs to be sensitive and specific, or else it will only serve to add yet another piece of conflicting clinical information to an already complex and confusing medical picture. In simpler terms, a biomarker can be considered as a laboratory test that is readily available and can accurately point to a clinical condition of interest, such as an infection or sepsis. Unlike ordinary sepsis, which annually affects roughly 750,000 Americans who are more likely to be elderly...
or have predisposing medical comorbidities, sepsis following trauma occurs in a younger cohort who typically have far fewer comorbidities. Outwardly, this observation would suggest that diagnosing sepsis in trauma patients ought to be easier and that a trauma biomarker would be readily available; however, the physiologic responses to injury are often indistinguishable from sepsis and therefore render this assumption invalid.

Traumatic injury spans a continuum from minor localized injuries to overwhelming severe polytrauma affecting nearly every organ and tissue in the body. Hemorrhagic shock and subsequent resuscitation expose the individual to whole-body ischemia-reperfusion injury, can incite the activation of multiple cell lines and generation of numerous proinflammatory and antiinflammatory cytokines, and prime the patient for additional physiologic insults or hits that can produce an exaggerated physiologic response.\(^6,7\) Even a relatively moderate injury such as a closed femur fracture typically results in the systemic inflammatory response syndrome (SIRS).\(^8\) Thus, tachycardia, tachypnea, leukocytosis, and low-grade fever following such an injury are part of the normal physiologic response to injury rather than signs of infection. Therefore, the classically accepted biomarkers such as temperature, white blood cell count, heart rate, or respiratory rate following trauma are greatly diminished in their ability to indicate infection. In this confusing setting of both normal and abnormal signs and conflicting clinical data, a trauma biomarker should stand out as a sensitive yet specific marker of infection.

Because inflammation following trauma is the norm, some investigators have attempted to quantify the degree of inflammation, the time it takes to return to baseline, or late spikes in inflammatory markers as predictors of organ failure or death. Much of this work has focused on the early inflammatory (T-helper cell [Th]1) cytokines such as IL (interleukin) 1, IL-6, IL-8, and tumor necrosis factor (TNF),\(^9,14\) but the preponderance of the literature supports IL-6 as the biomarker of choice in trauma.\(^15\) IL-6 is a ubiquitous, multifunctional, regulatory cytokine that is important to initiating the acute phase response in an organism. IL-6 plasma levels have been shown to correlate with the severity of injury\(^16–20\) and can predict complications such as pneumonia\(^21\) and even death\(^20\) as a result of major trauma. Despite some of the promising results with IL-6 as a biomarker, it fails to fulfill many of the tenets required of an ideal biomarker because it is relatively insensitive, lacks specificity, and is not readily available to most bedside clinicians. The same limitations are true for the other inflammatory cytokines that are part of the Th1 inflammatory response and hinder their use as biomarkers.\(^22\)

Initially, most investigators focused on the early proinflammatory response to trauma and attempted to use these markers to predict outcome with little tangible success.\(^10–22\) Over time, it has become apparent that following traumatic injury, the immune system progresses from an initial hyperinflammatory state to a compensatory antiinflammatory state, resulting in profound immune suppression.\(^23\) It is not by coincidence that most late trauma deaths occur during this period of posttraumatic immune dysfunction, thus further demonstrating the need for an accurate biomarker in this state. Some of the more common mediators of the posttraumatic immune-suppressed state are IL-4, IL-10, prostaglandin E\(_2\) (PGE\(_2\)), and transforming growth factor \(\beta\) (TGF-\(\beta\)).\(^24\) Among these mediators, IL-10 is perhaps the most important regulatory cytokine and has a major effect on T lymphocytes, causing their suppression.\(^24\) PGE\(_2\) is another important regulatory mediator and is responsible for macrophage downregulation and for suppressing the production of TNF and the inflammatory cytokine IL-12.\(^25\) Although all these mediators are important components of the immune-suppressed state, none of them are capable of functioning as an effective
biomarker, nor are they sensitive enough to discern infection against the background of generalized immune dysfunction.

Despite the tremendous advancements made in the understanding of the complex interactions between the innate and humoral immune systems, particularly as they relate to the responses to acute illness and injury, the definitive biomarker of infection in the confusing milieu of varying posttraumatic inflammation has not yet been found. With each new discovery of a putative mediator of trauma-induced hyperinflammation or hypoinflammation, it is hoped that the so-called Holy Grail or ultimate biomarker would be found. The search for the ultimate biomarker has taken an interesting turn as of late, with the growing concept of the “danger signal” model in immunology.26 According to this model, immune system activation is dependent on signals derived from damaged or dying cells, both foreign and endogenous, and trauma (and other similar stimuli) can result in SIRS in addition to the expected immunologic responses to infection. As noted previously, SIRS is the expected response to trauma and makes the distinction between infection and sterile sepsis exceedingly difficult.27,28 No clear-cut biomarker has emerged as a result of this paradigm shift in immunology, but there are several promising candidates that have been appropriately named the alarmins.29,30 The alarmins and other danger signals hold great promise as potential biomarkers, but the experimental and clinical data to support this optimism are limited.

There are many so-called endogenous danger molecules that are liberated as a result of tissue injury, including the intracellular chaperones or heat shock proteins, defensins, annexins, S-100 protein, cathelicidin, eosinophil-derived neurotoxin, and high mobility group box nuclear protein 1 (HMGB1).29–31 Among these molecules, HMGB1 is emerging as a leading biomarker of tissue injury and by default, an ostensible trauma biomarker. HMGB1 is an intracellular protein than can translocate to the nucleus where it binds to DNA and regulates gene expression; however, under certain pathologic conditions, such as those that cause cellular necrosis, it can be released into the bloodstream where it has different immunomodulating properties.32 HMGB1 is a true danger signal in that its most powerful effects on the immune system occur when it is released in response to cellular necrosis and not apoptosis.32 Thus, unregulated cell death is a far more potent trigger for the role of HMGB1 in the immune system than programmed or apoptotic cell death. Plasma HMGB1 levels have been shown to correlate with mortality in both sepsis33 and trauma.34 Although data are limited, HMGB1 levels seem to correlate with the severity of injury, tissue hypoperfusion as measured by base deficit, and levels of other inflammatory cytokines such as IL-6, as well as with the likelihood of developing multiple-organ dysfunction syndrome and death following trauma.34 In this regard, HMGB1 shows great potential as an overall prognostic indicator after trauma, but its ability to predict infectious complications of trauma remains undetermined at present.

The role of HMGB1 in stratifying mortality in septic patients is not clear, with some conflicting studies from humans and animals showing that it may or may not correlate well with the likelihood of dying of sepsis.35,36 HMGB1 may be a much better candidate as a trauma biomarker than as a biomarker for critical illness or sepsis because its expression seems to be at the highest following hemorrhagic shock.37 Indeed, experimental animal data further indicate the importance of HMGB1 in hemorrhagic shock, because blocking HMGB1 in vivo results in decreased organ failure and mortality caused by hemorrhage.38,39 Whether HMGB1 plays a role in susceptibility to bacterial infection and sepsis following hemorrhagic shock remains to be demonstrated in humans, but animal data suggest that it is intricately associated with inflammation, following a “2-hit” model of hemorrhage followed by bacterial challenge.40
Out of the myriad of potential biomarkers of posttraumatic sepsis and death, the current leading candidate would have to be procalcitonin (PCT). PCT is the peptide precursor of the hormone calcitonin, which is normally produced by parafollicular C cells of the thyroid and is important to calcium homeostasis.\(^41\) However, in response to bacterial infection, PCT is released from various cell types outside the thyroid and has been shown to be a reliable indicator of bacterial infection and sepsis.\(^42,43\) PCT levels have been shown to be elevated in septic patients who have undergone total thyroidectomy, and elevated PCT levels do not subsequently result in the elevation of serum calcium levels.\(^44\) PCT seems to be an intermediary marker of infectious inflammation because its levels peak 3 to 4 hours after an infectious insult, whereas the cytokine IL-6 and TNF are produced in the first hour or so.\(^45\) Thus, PCT secretion is dependent on IL-6 and TNF, but the cytokines do not seem to be appreciably affected by PCT release.\(^45\)

PCT’s importance to the septic response is borne out by evidence showing that it is a reliable early indicator of severe postoperative complications\(^46–48\) and that its levels correlate with the incidence and severity of multiorgan failure.\(^49,50\) It is not uncommon for PCT levels to increase by a factor of more than 1000 times in response to endotoxin or inflammatory cytokine exposure. Because the half-life of PCT is roughly 22 hours, a decrease in its level can also be viewed as a biomarker of resolving infection, which is another characteristic that adds to its overall utility as a biomarker. Serial sampling of PCT after trauma has revealed that PCT levels are typically elevated shortly after traumatic injury, but this elevation typically subsides unless a subsequent bacterial infection occurs.\(^51\) Elevations in PCT levels immediately after traumatic injury seem to predict that the patient is at increased risk for sepsis during hospitalization and should prompt an anticipatory approach to infection in that patient.\(^51\)

Because PCT levels are intimately related to the presence (or absence) of bacterial endotoxin in the circulation, it is a far more sensitive biomarker of infection than nonspecific markers, such as C-reactive protein (CRP).\(^52,53\) CRP belongs to the family of acute phase reactants, is synthesized primarily in the liver and lung, and is important to the function of complement and for the opsonization and phagocytosis of bacteria.\(^54\) IL-6, which is predominantly released by macrophages, promotes the release of CRP in response to virtually all sources of systemic inflammation, including infections or trauma.\(^55\) Before the advent of more sophisticated biomarkers such as PCT, CRP was the most extensively investigated biomarker in critically ill patients. CRP was used in establishing a diagnosis of sepsis, because of its prognostic ability in septic patients, and as an indicator of the severity of sepsis.\(^5\) CRP levels are easily determined and reliable; commercially available assays can rapidly determine the serum level of CRP. However, when compared with PCT, CRP is far less sensitive, thus limiting its role as a useful biomarker for infection following traumatic injury. Head-to-head comparisons and analyses have borne this out, thus further supporting PCT, rather than CRP, as a leading biomarker for infection following traumatic injury.\(^56\)

PCT has another valuable role in the care of trauma patients besides its ability to indicate bacterial infection that seems incongruent with its correlation to bacterial infection. Several investigators have shown that PCT levels spike shortly after major traumatic injury and that the magnitude of this level correlates with the propensity to develop multiorgan failure.\(^57–59\) Elevated PCT levels following trauma have been shown to coincide more with blunt abdominal trauma than trauma to other body regions.\(^60,61\) One possible explanation for the observed linkage between abdominal trauma and elevation of PCT levels centers on a direct effect on the bowel itself, breaching gut barrier integrity and allowing luminal bacteria to gain access to the bloodstream. Alternatively, traumatic shock, particularly hemorrhagic shock, often...
results in bacteremia or endotoxemia unexplained by direct injury to the gastrointestinal tract and has been cited as evidence for the controversial concept of gut barrier failure and bacterial translocation.62–64 Whatever the inciting stimulus is, the association of PCT with abdominal trauma lends further credence to its role as a trauma-specific biomarker and as a predictor of organ failure and bacterial infection following major traumatic injury.

Other gut-derived substances have been identified and have many similarities to PCT both in terms of the predictive ability as a biomarker and in the pattern of release following traumatic injury. Pancreatic stone peptide/regenerating peptide (PSP/reg) was initially discovered in the pancreas but was then subsequently detected in the bloodstream of patients with severe pancreatitis.65 PSP/reg is a lectin-binding acute phase protein, whose release is promoted by IL-6. The protein has been isolated from several extrapancreatic tissues.66 PSP/reg is incredibly similar to PCT in that its low-level constitutive expression from its native tissue is vastly upregulated in response to the inflammatory cytokines of the septic response, and this induced expression occurs in tissues outside the site of its constitutive expression. Once in the bloodstream, PSP/reg causes the activation of leukocytes and has been shown to be a sensitive indicator of sepsis.67 In a study comparing PCT with PSP/reg in moderately to severely injured trauma patients, PSP/reg seemed to be a better sign of infectious complications in the confusing hyperinflammatory postinjury state. In addition, PSP/reg shows some ability to differentiate between localized and systemic infection.67 The high signal-to-noise ability of PSP/reg for sepsis coupled with its capacity to discern local from systemic infection renders it an encouraging trauma biomarker, but like all biomarkers, more data are required to truly identify its proper role.

Emerging data about cellular biomarkers such as peripheral endothelial progenitor cells (EPCs) also hold great promise for the role of these biomarkers in clarifying the immune state following severe trauma. EPCs have shown some ability to make the straightforward distinction between SIRS, sepsis, and severe sepsis because their levels seem to closely parallel this infectious continuum. EPCs are released from the bone marrow in response to infection and have shown correlation with the severity of sepsis; thus, they should serve a useful role as a trauma biomarker, although the timing of the appearance of EPCs in response to infection remains to be elucidated.68 Certainly, EPCs warrant further investigation as a sepsis biomarker in trauma patients, but it is unclear what their true utility will be in light of trauma’s profound negative effect on the function and cellular components of the bone marrow.69

A second cellular biomarker is CD4⁺CD25⁺ regulatory T (Treg) cells, which are responsible for controlling the immune response to infection. Under normal conditions, Treg cells comprise no more than 5% to 10% of the circulating CD4⁺ T cells, but their levels are greatly elevated in septic patients but not in those with SIRS. Like EPCs, Treg cells may be useful biomarkers in the complex and changing posttraumatic immune state, which should prove particularly useful in caring for survivors of the most severe trauma.70 However, because Treg cell activity is dependent on direct cellular interactions that seem to be partially mediated by TGF-β, it is much more likely that Treg cells are powerful effectors of the postinjury immunocompromised state rather than true symbols of infection.71 It seems that Treg cells are partly responsible for the immune paralysis that follows many shock states and probably contribute to T-cell anergy as well, but these activities alone are probably not suitable for their role as a biomarker, other than to confirm the postshock immunocompromised state.72

Recently, yet another promising biomarker that shares many of the positive traits of PCT and PSP/reg has been identified. This new biomarker is the N-terminal fragment
of the precursor of C-type natriuretic peptide (CNP), or NT-proCNP. The natriuretic group of peptides is a diverse group of proteins that are released by the heart and central nervous system and have various important physiologic properties. Atrial natriuretic peptide (ANP) is produced by atrial myocytes, and brain (or B-type) natriuretic peptide (BNP) is predominantly produced by ventricular myocytes. Both ANP and BNP are released in response to cardiac-wall stretch, foment natriuresis and diuresis, and oppose the action of many inflammatory and sympathetic neuroendocrine mediators.

CNP was initially identified in the central nervous system but has subsequently been localized to the endothelium of the vascular system. CNP release from the endothelium is driven by most of the inflammatory cytokines of the TH1 response, such as IL-1 and TNF, and CNP functions to preserve the flow through the vasculature by opposing the action of the potent vasoconstrictors endothelin 1 and angiotensin II. Both ANP and BNP serve as potent stimulators of CNP release, but unlike ANP and BNP, CNP has essentially no effect on natriuresis, diuresis, or the renin-angiotensin system. Because the inflammatory septic cytokines promote CNP release from the endothelium, CNP’s precursor NT-proCNP should be a potential biomarker of infection. Indeed, continuous sampling of NT-proCNP in severely injured trauma patients showed a dramatic increase in its level nearly 2 days before the development of a septic event. Enthusiasm for use of NT-proCNP as a posttraumatic marker of infection must be tempered by the fact that traumatic brain injury, which commonly occurs after blunt trauma, may dramatically affect CNP and NT-proCNP levels in response to septic insults, thus affecting its sepsis predicting abilities.

The ongoing search for the ideal biomarker in trauma patients is beginning to be successful as more sensitive and specific indicators of the posttrauma immune state emerge. Confounding this search is the fact that the normal physiologic response to major injury is immune activation followed by immune suppression, both of which have attributes mirroring the septic condition. A reliable biomarker is desperately needed for severely injured trauma patients because they are at increased risk for sepsis and sepsis-related mortality. In fact, in some studies, these patients have a mortality rate approaching 40%.

Clinical work relating to sepsis biomarkers remains in the earliest stages, but even at this early juncture, the number of would-be biomarkers is astounding. To date, there have been more than 3300 publications involving nearly 180 different sepsis biomarkers, but the leading trauma biomarker candidates can be narrowed to PCT, PSP/reg, and NT-proCNP. Remarkably, all 3 of these putative biomarkers share similar traits such that all 3 are constitutively expressed by 1 cell type but are radically upregulated and released from different cells in response to a bacterial infection. Of these 3 biomarkers, PSP/reg and NT-proCNP seem especially promising candidates for the Grail because they seem to be more sensitive than PCT for infection following trauma, but it is not known yet whether these biomarkers will stand the test of time as the clinical and scientific data surrounding their use grow.

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