Inflammatory serum markers in patients with multiple trauma

CAN THEY PREDICT OUTCOME?

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Clinical trials have provided information concerning the various immunological alterations that occur after trauma which may cause the multiple organ dysfunction syndrome (MODS) and death. Once multiple systems are dysfunctional the rates of mortality are as high as 50%, the morbidity is severe and the costs of health care are enormous. Although external support such as ventilators, dialysis machines and inotropic drugs, is provided to all patients to compensate for the internal dysfunction until they can support themselves, the end result is often unpredictable.

Early evaluation of the prognosis in polytraumatised patients is difficult. Traditionally, the clinical condition and management of the patient are assessed by evaluation of cardiovascular, renal, liver and respiratory functions. Nevertheless, the significance of such clinical parameters as the urinary output, oxygen saturation, blood gases, C-reactive protein, base excess, etc., is limited since only patients with a clearly impaired organ function can be distinguished. Organ dysfunction which is not detectable by these parameters does not guarantee a condition of the patient which is stable enough for operation especially if in the ‘borderline condition’.

The predictive value of several clinical parameters is uncertain. For instance, initial levels of lactate have been shown to correlate with the development of MODS and prolonged hyperlactataemia is associated with increasing mortality. By contrast, Rixen et al. demonstrated that the level of lactate was not sufficiently sensitive to predict the probability of death but that age, the Glasgow Coma Score (GCS), the Injury Severity Score (ISS), base excess (BE) and the prothrombin time were the most important predictors of the development of post-traumatic complications and death.

However, Tremblay, Feliciano and Rozycki reported that the value of BE depends on the mechanism of injury since it appears to be useful in assessing patients who have sustained blunt trauma, but not in those with a penetrating injury. It was reported to be particularly ominous in patients older than 55 years, without manifestation of base deficit in significant injuries.

With advances in molecular medicine, new information has emerged regarding the response to injury and shock at the molecular level. Several mechanisms have been proposed for the development of post-traumatic complications including the macrophage theory, the gut hypothesis, the two-hit theory and the micro-environment theory.

As a result of the popularity of the micro-environment theory and the availability of techniques to measure molecular mediators, studies have been undertaken to search for inflammatory markers which could detect patients in the ‘borderline condition’ and at risk of developing post-traumatic complications. Alteration of treatment may then prevent the onset of adverse sequelae.

The purpose of this review is to highlight our current knowledge on the effectiveness of the existing inflammatory markers of immune reactivity and evaluate their impact on our clinical practice.

Molecular aspects of trauma

Under normal physiological conditions and after surgical or accidental injury all cells in the body constantly communicate with each other.
An array of regulatory proteins produced and secreted by lymphocytes and other cells have a role in cascading the immune response to trauma. Following trauma, patients are subjected to dynamic alterations in the haemodynamic, metabolic, and immune responses which are largely orchestrated by endogenous mediators referred to as cytokines. An injured or a surgical patient is a ‘stew’ of pulsating cytokines.

The development of this inflammatory response which may have fatal consequences is part of the normal response to injury. This gives rise to the systemic inflammatory response syndrome (SIRS) followed by a period mediated by a compensatory anti-inflammatory response syndrome (CARS). Within this inflammatory process there is a fine balance between the beneficial effects of inflammation and the potential for the process itself to cause remote tissue injury leading to the adult respiratory distress syndrome (ARDS) and MODS.

According to the ‘one-hit’ model the initial massive injury and shock give rise to an intense systemic inflammation which causes activation of the innate immune system, including macrophages, leukocytes, natural killer cells and inflammatory cell migration enhanced by the production of interleukin (IL)-8 and complement components (C5a and C3a). In the ‘two-hit’ model, the stimulus is less intense and normally resolves, but the patient is vulnerable to secondary inflammatory insults which can reactivate SIRS and precipitate late MODS. The second insult may take many forms, among them sepsis and surgical operations. The clinician may have to make difficult decisions as to when and how much to do for the borderline patients with polytrauma. Hyperstimulation of the inflammatory system, either by single or multiple ‘hits’, is considered by many to be the key element in the pathogenesis of ARDS and MODS.

Numerous studies have shown that stimulation of a variety of inflammatory mediators takes place immediately after trauma. This response initially corresponds to the first-hit phenomenon. Several investigators have also highlighted the issue of secondary surgical procedures acting as additional inflammatory insults or second-hit phenomena. These models of first- and second-hit biological responses to different stimuli have now become the basis of our treatment plans and investigations. At the molecular level, a variety of inflammatory mediators has been implicated in the pathogenesis of organ dysfunction. Serum markers of immune reactivity can be selectively grouped into markers of acute-phase reactants, mediator activity, and cellular activity (Table I).

### Markers of acute-phase reactants

This group of markers is usually of hepatic origin and includes the lipopolysaccharide-binding protein (LBP), procalcitonin and C-reactive protein.

**Lipopolysaccharide-binding protein (LBP)**. This is a 58kDa class-I acute-phase protein of mainly hepatic origin with the ability to bind bacterial lipopolysaccharide (LPS). LPS-activated macrophages release proinflammatory cytokines, such as IL-6, IL-1 and tumour-necrosis-factor (TNF)-α. LBP is synthesised in hepatocytes and released into the blood. During the acute-phase response, this synthesis and release can increase up to 30-fold. LBP was found to block the effects of LPS in vitro and protect mice from lethal outcome. LPS-induced production of cytokines by macrophages was decreased by high concentrations of LBP. Increased levels of LBP also prevented liver injury and reduced mortality after an injection of LPS in mice. However, low concentrations of LBP enhanced production of TNF-α in macrophages induced by LPS. In general terms, LBP enhances the effects of LPS when it is present in small quantities, whereas it suppresses them in high concentrations.

Levels of LBP rise in patients during the acute phase of trauma or sepsis, with maximum values occurring on the second and third days. In patients with MODS significantly higher concentrations of LBP were found in those with documented infection. By contrast, no significant differences were found between patients with SIRS and the non-septic MODS group, suggesting that LBP may be used as a marker to differentiate between SIRS and ongoing bacterial sepsis in the early post-traumatic course.
In patients with severe sepsis or septic shock, concentrations of LBP were found to be significantly increased and it may have prognostic significance in such patients although Blairon, Wittebole and Laterre reported that LBP is only a non-specific marker for sepsis, and that the response was not clearly correlated with the severity of infection.

**C-reactive protein (CRP).** This is one of the acute-phase response proteins produced by hepatocytes and is usually found in concentrations of 0.3 to 1.7 mg/ml. Increased production is due to cytokine-dependent induction of synthesis and elevated levels may be detected within eight hours of a stimulus and can reach 500 mg/l. Besides trauma, elevated levels of CRP may be seen in other conditions such as autoimmune disease, infection and malignancy. The level of CRP normally peaks within 48 hours of the stimulus. A fall in serial measurements usually indicates resolution of the underlying process, while persisting elevated levels may indicate ongoing inflammation or infection.

While the level of CRP has been used in clinical practice for many years, it is relatively non-specific and not predictive of septic complications after major trauma. Clinical studies have shown that there is no correlation between the serum CRP and the severity of injury or prediction of survival in polytraumatised patients. In patients who are critically ill neither the absolute level of CRP nor the rate of its change has been found to relate to proven infection. Despite widespread clinical use, the level of CRP is not considered to be an ideal marker of the inflammatory mediator response after trauma.

**Procalcitonin (PCT).** This is derived from a precursor protein preprocalcitonin, proteolysis of which results in the formation of calcitonin. The latter is normally produced in the C-cells of the thyroid. PCT is not normally detected in the plasma of healthy individuals. A study of hepatocyte tissue culture treated with TNF-α or IL-6 has shown detectable levels of PCT after 24 hours of culture, suggesting that the liver is a potential source of production of PCT.

In polytraumatised patients injury leads to increased plasma levels of PCT dependent on the severity of injury, with peak values on the first and third days. Increased concentrations of PCT during the first days after trauma have been shown to predict severe SIRS, sepsis and MODS. Thus, PCT may be a useful marker for monitoring the inflammatory status in these patients. There are significantly increased plasma levels of both PCT and IL-6 in males compared with females.

In a prospective study of 175 patients admitted to a surgical intensive-care unit the correlation between TNF-α, IL-6 and various markers of inflammation was determined. PCT proved to be the best of these. In contrast to the concentration of CRP a secondary increase in that of PCT seemed to be an adequate indicator of severe infection during late SIRS. However, not all studies have shown PCT to be better than CRP as a marker of infection. In a study of 205 medical and surgical patients a cut-off level was described for the diagnosis of infection as 0.6 ng.ml\(^{-1}\) for PCT and 7.9 mg.dL\(^{-1}\) for CRP. The latter showed a higher sensitivity (71.8% vs 67.6%) and specificity (66.6% vs 61.3%) for infection. Confirming these findings, a study of 60 patients showed a higher level of PCT in infected than in non-infected patients but the level of CRP was more predictive of infection.

Post-operative plasma concentrations of PCT in patients without signs of infection are largely influenced by the type of surgical procedure. It has been shown that during the first two post-operative days, the concentration of PCT is elevated in all patients. Higher levels are found after major surgical procedures compared with minor operations. In severe lung contusion the level of PCT in bronchoalveolar lavage (BAL) was not considered to be a reliable parameter for assessing the extent of lung contusion.

In general terms, the absolute level of PCT and the cut-off points for diagnosis appear to vary significantly, and the prognostic value of PCT in trauma is as yet unclear. However, the correlation between PCT and the severity of sepsis appears to be constant in all the available studies.

**Markers of mediator activity**

**Tumour necrosis factor (TNF).** This is an autacoid which exists in multimers of two or three identical subunits and contains several potential sites of glycosylation. TNF-α and TNF-β may be distinguished. Both have almost the same biological effects. TNF-β is only synthesised by lymphocytes. In inflammatory reactions, TNF-α is mainly involved. It may also exist as an integral membrane protein. Membrane bound TNF-α is a 26kD polypeptide which includes a residual signal sequence, and is usually cleaved to yield the soluble form. TNF-α is a central regulator in the immunoinflammatory response after trauma and is produced by monocytes, lymphocytes, Kupffer cells, macrophages, endothelial cells and glial cells. It has a short half time in the plasma of 14 to 18 minutes and is cleared by binding to soluble TNF-α receptors and natural TNF-α binding proteins. Release of TNF-α is stimulated by such triggers as IL-1, 2 and 12, interferon-γ, platelet aggregating factor (PAF), complement protein C5a as well as by endotoxing and can induce its own release. Stimulation of TNF-α can be down-regulated by IL-4, 10 and 13, TNF-α, cortisol and agents which increase intracellular cyclic adenosine monophosphate.

The effects of TNF-α on endothelial cells are diverse. It increases both the permeability of endothelial cells and the expression of adhesion molecules like intercellular adhesion molecule 1 (ICAM-1) or E-selectin, leading to the activation and adhesion of granulocytes. It also increases the procoagulated activity of endothelial cells.

Most of the available studies of TNF on patients with multiple injuries have been focused on the clinical course of patients in intensive-care units. Despite the initiation of treatment continually high levels of TNF-α have been reported to correlate with a poor outcome, although there was no significant difference in the levels of TNF-α when
study commenced. This is not a consistent finding since high levels of TNF-α have been associated with increased survival in patients with septic shock secondary to intra-abdominal pathology. In patients with meningococcal meningitis serum levels of TNF-α have been shown to correlate with mortality but other authors have failed to show significant increases in TNF-α in septic patients. The results of using TNF-α as a marker of sepsis and a predictor of mortality have been disappointing. This is partly due to the pharmacokinetics of TNF-α; single-point measurements have proved to be unhelpful since peak concentrations of TNF-α are reached in one or two hours and may have significantly decreased by four to six hours. Somewhere between these times systemic signs of sepsis occur. By the time a septic event is recognised, concentrations of TNF may have returned to the baseline level. The origin of the sepsis also determines the magnitude of changes in circulating TNF. Increased concentrations of TNF have also been observed in BAL of polytraumatised patients with post-traumatic ARDS.

Efforts to modify the effect of TNF-α have included the use of anti-TNF-α antibodies and the use of recombinant soluble TNF-α receptors. In a rodent model, mortality after an injection of endotoxin was significantly decreased by pretreatment with TNF-α antibodies. In another experimental study, applications of TNF-α antibodies prevented the development of organ dysfunction in E. coli sepsis. However, application of TNF antibodies before the induction of experimental peritonitis resulted in increased rates of mortality. Furthermore, the antibody approach has produced little evidence of benefit in man although improvements in the haemodynamics of patients have been observed. The use of recombinant soluble TNF-α receptors has been shown to improve survival in mice, but at present there have been no similar results in man. So far the use of TNF-α or its soluble receptors has not proved to be useful as a diagnostic marker.

Interleukin-1 (IL-1). This family of peptides consists of three structurally related polypeptides: IL-1α, IL-β and IL-1 receptor antagonist (IL-1ra), produced primarily by monocytes. It is primarily induced in the presence of ischaemia or sepsis by activated macrophages and activated endothelial cells. Biologically, IL-1 has a similar activity to that of TNF-α and acts synergistically with it with induction of fever, hypotension, endothelial cell adhesion as a procoagulant, and the chemotaxis of polymorphonuclear leucocytes (PMNs) and macrophages. In addition, IL-1 induces release of TNF-α, IL-6, IL-8, platelet activating factor (PAF) and eicosanoids. The circulating half-life of IL-1 is six minutes. This makes its detection after injury much less likely than that of TNF-α. Most studies carried out to assess the efficacy of this marker have been done in septic patients in whom it was found that the levels of IL-1β did not correlate with death or MODS.

IL-1ra is a cytokine-like molecule which binds to the IL-1 receptor but does not induce a response. Concentrations of IL-1ra increase significantly in volunteers exposed to endotoxin and concentrations are higher in survivors than in non-survivors of animal models of sepsis. It has been given to patients with SIRS and the clinical picture of septic shock. In an initial trial in 99 patients, but not in a subsequent phase-III trial, those receiving IL-1ra showed a dose-dependent improvement in their clinical course and rates of mortality. This improvement correlated with a reduction in levels of IL-6 consistent with the control of IL-6 by IL-1 and the correlation of the severity of the disease and outcome with levels of IL-6. Perhaps because of the dynamic interaction between IL-1 and IL-1ra, there is no clear relationship between the levels of IL-1 and the severity of sepsis. This limits its usefulness as a clinical indicator or in research.

Interleukin-6 (IL-6). This is a glycoprotein with a molecular weight of 22 to 29 kD. It is produced by a variety of cells including T- and B-cells and endothelial cells. Production of IL-6 is induced by viruses, LPS, IL-1 and TNF. It induces a proliferation of B-lymphocytes with increased synthesis of immune globulins and a proliferation of T-lymphocytes. Furthermore, IL-6 causes an enhanced differentiation of cytotoxic T-cells and an increased activity of natural killer (NK) cells. Its main effect is to induce hepatic synthesis of acute-phase proteins such as CRP, fibrinogen, α1-antitrypsin or complement factors.

IL-6 is less transient and therefore more readily measurable than either IL-1 or TNF-α. It appears to be one of the best prognostic markers regarding the outcome of patients with SIRS, sepsis or MODS. The association between early increased plasma levels of IL-6, high ISS and a late adverse outcome has been well documented in several studies. An early marked elevation of the concentration of IL-6 (>500 pg/dl) will distinguish injured patients who later develop MODS. Hack et al. reported a direct correlation between IL-6 and levels of lactate in septic shock and IL-6 appeared to have prognostic significance in the differentiation of survivors from those who died (p = 0.0003). Increased concentrations of IL-6 in BAL were associated with a poor outcome in patients with ARDS. Dofferhoff et al. found a correlation of IL-6 with severity of illness in septic patients as measured by the APACHE II score, but there was no correlation between IL-6 and outcome. In trauma patients, IL-6 is not predictive of septic complications but rather correlates with the magnitude of the injury. It appears to be a marker of traumatic insult, with significantly elevated levels occurring within one to four hours after trauma, and correlates with the severity of illness.

Interleukin-10 (IL-10). This is an 18 kD protein mainly synthesised by T-lymphocytes but also produced by B-lymphocytes, monocytes and macrophages. Liberation of IL-10 is at least partially induced by TNF. It is an anti-inflammatory cytokine and reduces the synthesis of TNF-α and IL-1 by mononuclear cells after application of endotoxin, both in vitro and in vivo. Therefore it is also called a macro-
phage-deactivation factor. It decreases cytokine production of Th1 cells, which synthesise IL-2, IL-3, IFN-γ and TNF-β. These cells induce a type-IV immune reaction, a delayed type of hypersensitivity reaction (DTH), via cytotoxic T-cells while cytokines of the Th2 clone cause a humoral immune response. Moreover, IL-10 inhibits both the antigen-presenting function of macrophages and the subsequent proliferation of T-cells.68

The plasma concentrations of IL-10 are elevated in patients with polytrauma69,70 and after major surgery71 and a correlation with the severity of the injury has been reported.

An increased plasma concentrations of IL-10 has been observed in septic patients and even higher values have been found in patients who had suffered septic shock.72 However, other studies have shown an unchanged secretion of IL-10 in patients undergoing major surgical procedures73 and even depressed concentrations in trauma patients.74

In animal studies neutralisation of IL-10 by specific antibodies increased the rates of survival after burns and subsequent sepsis.75 In a sepsis model, the neutralisation of IL-10 reduced the vulnerability to secondary infections such as Pseudomonas pneumonia.76 In a peritonitis model, the early blockade of IL-10 by antibodies led to elevated concentrations of TNF in the plasma and increased rates of mortality. In the same model, both the therapeutic and the prophylactic application of IL-10 reduced the early liberation of the proinflammatory cytokines IL-1, IL-6 and TNF-α.77 A later neutralisation of IL-10, 12 hours after the induction of sepsis, seemed to be beneficial for survival.77 In another study, application of IL-10, six hours after the induction of peritonitis, improved the rates of survival in a murine model.78 However, Remick et al79 reported no significant decrease of the rate of mortality after the application of human recombinant IL-10.

While the above studies support the CARS hypothesis,80 it is as yet not clear whether early application of exogenous IL-10 reduces mortality. The results appear to be inconsistent even in animal studies.

Interleukin-18 (IL-18). This was formerly termed interferon-γ (IFN-γ)-inducing factor, and shares structural features with the IL-1 family. It amplifies the development of Th1 cells and it is an important factor for enhancing the activity and proliferation of NK cells.81

IL-18 is primarily secreted by activated macrophages, dendritic cells and Kupffer cells.82 It is synthesised as an inactive precursor protein which is processed into the mature form by IL-1β-converting enzyme/caspase-1 (ICE).83 Secretion of active IL-18 into whole blood from healthy humans and trauma patients can be effectively controlled by blockade of the activity of ICE.84 By contrast, during sepsis, alternative mechanisms are supposed to regulate the secretion of IL-18 since ICE inhibitors do not influence the release of IL-18 into whole blood and septic patients.84,85

Most studies which characterise the expression of IL-18 have been performed in septic patients.86,87 Hochholzer et al88 found in an experimental study that IL-18 played an important role in the induction of IFN-γ and lethality in response to LPS. Values of IL-18 were significantly correlated with their APACHE II scores in septic patients and a strong correlation between these and the levels of inflammatory cytokines was observed.89

In a clinical study in patients with post-operative sepsis, the level of IL-18 was significantly higher in both survivors and non-survivors than in the non-septic control group.86 It was also observed that the level was significantly increased in patients with lethal sepsis compared with septic survivors, with the conclusion that serum IL-18 represents an early predictive factor for the lethal outcome of post-operative sepsis. The release of IL-18 seemed to depend on the presence of micro-organisms because trauma-induced systemic inflammation without circulating bacteria did not increase the plasma concentration of IL-18.84,85

Markers of cellular activity

Cytokine receptors. Cytokines exert their effects by interaction with receptor systems and mediate intercellular events which often involve transcription of DNA.90

The effect of TNF-α is mediated by the binding to two membrane-bound receptors (TNF-RI, 55kD and TNF-RII, 75kD), which are found on nearly all cell types. In polytraumatised patients, increased concentrations of membrane-bound receptors were found to correlate with increased rates of MODS.91 Each TNF receptor has a corresponding soluble molecule (sTNF-RI and sTNF-RII), which competes with membrane receptors for binding free TNF-α, thus eliminating the bioactivity which would occur with membrane-bound receptor ligation. In a clinical study, early post-traumatic MODS and SIRS coincided with an increase in the concentration of soluble TNF receptor protein.92 In severely burned patients, an early increase in sTNF-RI and sTNF-RII was reported to be associated with a higher risk for poor outcome.93 Major surgery resulted in elevated sTNF-RI and sTNF-RII expression and high sTNF-RII levels were associated with increased post-operative rates of mortality.94,95 It was concluded that high levels of antagonists to TNF-α represented persisted activation of SIRS in critically ill patients.94,95

IL-1α and IL-1β bind to two classes of cell-surface receptor (IL-1-R1 and IL-1-R1I). It has been postulated that IL-1-R1I prevents IL-1 from binding to type-1 receptors, thus inhibiting signal transduction.96 IL-1 bioactivity is also inhibited by the presence of IL-1 receptor antagonist (IL-1ra). This inhibits IL-1 by binding to IL-1-R1I without agonist activity.96 Again, each membrane-bound IL-1 receptor has a soluble form (sIL-1R-I and sIL-1R-II). In polytraumatised patients, increased levels of IL-1ra were correlated with post-traumatic MODS.95 Mokart et al97 showed that raised concentrations of IL-1ra were associated with post-operative shock.

There are two forms of the IL-6 receptor, one soluble (sIL-6-R) and the other membrane-bound (mIL-r-R). IL-6...
bound to sIL-6-R exerts its function independently from mIL-6-R. In a clinical study, it was shown that serum levels of sIL-6-R remained in the normal range after severe head injury. They were elevated in the CSF, but no correlation with the extent of the cerebral lesion or the clinical outcome was observed. Serum concentrations were significantly higher than those in the CSF and a pivotal role for the IL-6/sIL-r-R ratio in the injured brain was suggested. After major thoracoabdominal surgery, serum levels of sIL-6-R were found not to be influenced by surgical trauma. Concentrations of sIL-6-R in the drainage fluid were significantly lower compared the serum levels. It was concluded, that sIL-6-R is being constantly produced in areas other than the operative field, while the level of sIL-6-R is reduced by consumption (binding to IL-6) in the operative field.

**Adhesion molecules.** The adhesion of polymorphonuclear leucocytes (PMNs) to capillary endothelial cells is the decisive step for their migration to the site of inflammation and the hallmark of the ‘microenvironment theory’. The adhesion is provided by adhesion molecules, which are present both on the PMN and on the surface of endothelial cells. Three major groups of adhesion molecule are distinguished: selectins, immune globulins and integrines. Both selectins and immune globulins also appear in a soluble form (sEselectin and sICAM-1).

All these proteins provide a specific attachment between the ligand and the receptor, so that a selective accumulation of PMNs occurs in the inflammatory tissue. The adhesion of PMNs to endothelial cells and the migration of PMNs through the endothelium are characterised by ‘rolling’, ‘attachment’ and ‘diapedesis’. These interactions of PMNs and endothelial cells are associated with different adhesion molecules. The neutrophil L-selectin and the endothelial P-selectin and Eselectin provide the so-called ‘tethering’ and ‘rolling’ of PMNs to endothelial cells. This transient adhesion between PMNs and the endothelial cells is associated with a lowered flow rate of PMNs and increased shearing forces. Furthermore, selectins are characterised by their adhesion being partially mediated via Lewis^s^ antigen and sulphate glycoconjugates. After activation of PMNs, a so-called ‘shedding’ of L-selectin appears. This soluble form of L-selectin (s-L-selectin) can be detected in blood serum. s-PMN-bound L-selectin was significantly reduced in deceased septic patients and in trauma patients with post-traumatic MODS. A blockade of L-selectin has been shown to exert protective effects in haemorrhagic shock.

After adhesion the PMNs are ‘attached’ to endothelial cells by a stable cell-to-cell contact via integrines. The integrin molecules consist of an α- and a β-subunit. The latter is subdivided in β1, β2 and β3, from which the β2-subunit is the most important. In addition, three different β2 integrines can be distinguished, CD18/CD11a (leucocyte function associated molecule-1, LFA-1), CD18/CD11b (macrophage antigen-1, Mac-1) and CD18/CD11c. In an *in vitro* model, the blockade of the common CD18 complex led to a significantly reduced adhesion of stimulated PMNs to activated endothelial cells. *In vivo* investigations in rabbits showed that bacteraemia-induced accumulation of PMNs in the lung was significantly lowered by a monoclonal CD18 antibody. Furthermore, the protective effect of this antibody was also demonstrated in different ischaemia-reperfusion models. The blockade of CD18 caused a significant reduction of accumulation of PMNs in a model of haemorrhagic shock, in mesenteric ischaemia in cats and in ischaemia of the limbs in the rat.

The third group of adhesion molecules are the immune globulins. Intercellular adhesion molecule-1 (ICAM-1) is the best known. ICAM-1 is mainly expressed by endothelial cells. Cells which have not been activated show a continuous baseline expression and a maximum expression occurs approximately eight hours after activation. Expression of ICAM-1 was shown to be induced by TNF-α, IL-β and endotoxin. ICAM-1 was identified as the endothelial ligand for β2 integrines.

Several studies have assessed the expression and kinetics of adhesion molecules in multiply-injured patients. While the levels of the CD11b receptor were elevated on admission, the degree of upregulation did not correlate with the ISS. However, levels of soluble Eselectin and soluble ICAM-1 were found to be significantly raised in the plasma by the third day after injury, the magnitude of the increase being related to the degree of injury. Law et al. reported a significant correlation between the elevated levels of soluble ICAM-1 and the later occurrence and severity of MODS. Post-traumatic MODS has also been associated with an increased number of CD11b/CD18 receptors on the leucocytes and an upregulation of the oxidative burst. In another study, both pulmonary endothelial cells and leucocytes showed a marked expression of ICAM-1 in patients who had died from septic complications. The levels of sICAM-1 also increased in sepsis and MODS, and death was predicted by serum concentrations of sICAM-1. However, it is not clear whether ICAM-1 is split off or secreted by endothelial cells since the soluble form of this molecule was also demonstrated in ICAM-1 knockout mice.

In general terms the available results in the literature are not consistent in assessing the predictive value of these molecules.

**Elastase.** Reactive oxygen species and proteases are neutrophil-derived toxic enzymes and have been considered to be important in the development of acute lung injury for some time. Mature neutrophils are unable to manufacture and store one such molecule, elastase, which has the capacity to degrade most proteins in the extracellular matrix and important plasma proteins. Besides its proteolytic activity, neutrophil elastase induces the release of pro-inflammatory cytokines (IL-6, IL-8). Under normal conditions the activity of neutrophil elastase is regulated by endogenous protease inhibitors. The release of neutrophil elastase, assessed as plasma elastase-α1 protease
inhibitor complex, is a sensitive marker of neutrophil cellular activity.

Significantly raised levels of E-α,PI have been noted on admission after multiple injuries and these were seen to be higher in those patients with greater degrees of injury and with the development of sepsis, being significantly elevated on the first and third days compared with patients without sepsis. One other study has reported a similar pattern of release of elastase but was unable to correlate this with the severity of injury, probably because of the small group of patients studied. Repetitive increases in the level of plasma elastase have been seen after trauma in patients who developed MODS. Nuytinck et al also noted that raised plasma levels of elastase correlated well with the severity of injury and the occurrence of multiple organ failure. Moreover, these authors were also able to discriminate between later survival or mortality at an early stage in the clinical course using this parameter.

More recent studies have also shown consistently that neutrophil elastase is increased in acute lung injury and in patients with ARDS with, in some cases, a positive correlation between the severity of illness, the respiratory index and organ failure.

The levels of neutrophil elastase are increased in both clinical and animal models of acute lung injury. However, there has been no validation of a cut-off point which could identify early patients at risk of developing post-traumatic complications. Further studies are needed to validate the efficacy of this marker of immune reactivity.

**Human leucocyte antigens; HLSA-DR class-II molecules.** In recent years, studies of the changes in the expression of some leucocyte cell-surface antigens early in the postinjury phase have been found to correlate with the development of subsequent septic complications and death. In particular, the level of expression of class-II major histocompatibility antigens (MHC class II) on mononuclear cells in the peripheral blood has correlated most consistently with septic morbidity and mortality after surgery or trauma. MHC-class-II antigens play an indispensable role in the presentation of processed antigen by antigen-presenting cells to T-cells for the elaboration of a specific immune response. Failure of this antigen-presenting capacity results in an abortive or non-existent response. This may indicate why some patients develop and succumb to septic complications after trauma, although it does not explain why some recover their monocyte HLA-DR expression whereas others suffering the same degree of trauma fail to do so, going on to develop septic complications and ARDS. Class-II major histocompatibility molecules are expressed on the surface of antigen-presenting cells, namely macrophages, dendritic cells and B cells.

In one study, when an initial low level of expression rose to normal levels within three to four days, the clinical course was uneventful. When the rise in MHC class-II expression was delayed, but still occurred, the post-operative course was complicated by the development of sepsis from which the patient recovered. However, when the level of monocyte MHC class-II expression failed to recover after trauma, patients often developed sepsis from which they died.

A study carried out in patients undergoing major resection of the digestive tract also showed that the operation was associated with a major fall in the level of expression of monocyte MHC class II, maximally on the first post-operative day, and that the pattern of recovery of this expression and the relationship to clinical outcome were similar to those seen after non-surgical trauma.

More recently, after multiple injuries, release of IL-10 was reported to regulate monocyte HLA-DR expression. In another study assessing the expression of HLA-DR on monocytes and T cells in 77 patients who had sustained blunt trauma, the HLA-DR on T cells was reduced on the day after admission in 20 patients with subsequent severe sepsis, compared with 40 who did not develop infection.

Several studies have described the delayed or complete failure of recovery of the expression of the HLA-DR antigen on circulating monocytes in patients destined to develop sepsis and who eventually died. The HLA-DR is considered to be the only marker of immune reactivity which correlates accurately with morbidity and mortality after trauma. However, the need for a dedicated laboratory for flow cytometric studies has not made the use of this marker very attractive in clinical practice.

**DNA.** The mechanisms by which cell-free DNA is liberated into the circulation of human subjects are unknown. One possibility is that DNA is released after cell death. Plasma DNA has been found to increase after major trauma and has also been suggested as a potentially valuable prognostic marker for patients at risk. In a clinical study in 83 multiply-injured patients the level of plasma DNA predicted organ failure and MODS with an overall sensitivity and specificity of 93% and 83%, respectively. In a further clinical study in 84 patients with multiple injuries, it was also confirmed that the plasma DNA was increased after trauma. The concentrations were 12-fold higher in patients with adverse outcome compared with those who did not develop such complications.

The prognostic value of free DNA has only recently been investigated. The existing preliminary studies are promising. Nevertheless, more studies are required to validate the sensitivity and specificity of such a marker in the clinical setting.

**Conclusions**

Understanding of the pathophysiology and the immunobiology of both traumatic and surgical injury have contributed considerably to the debate surrounding the aetiology of post-traumatic complications. Trauma and shock lead to an activation of multiple humoral and cellular cascade mechanisms, such as inflammatory mediators, as well as complex mechanisms of host defence. These immuno-
logical defence mechanisms may become insufficient and allow further complications. Generalised capillary damage, increase in permeability, and subsequent multiple organ dysfunction or failure belong to the clinical picture of MODS. Despite the application of conventional and supportive treatment in the intensive-care unit, the clinical results in patients with ARDS and MODS remain disappointing.135

Several attempts at inhibiting components of the mediator cascade to influence the outcome after trauma and sepsis have failed.136-138 The reason for this lack of success is because of poor understanding of the critical mechanisms, the difficulty identifying those patients who may benefit and the delay between the onset of complications and the administration of the therapeutic agent. Clearly, more evidence regarding the underlying biological problem is needed. This issue may be resolved by the use of inflammatory and molecular markers and genetic technology to allow the early identification of patients at high risk of developing complications.

However, for a marker to be clinically useful it must be easily and reliably measured, usually from a blood sample. Its usefulness is tested by examining its sensitivity and its specificity. An ideal marker will accurately discriminate between those with a given condition and those without. In the clinical setting, however, especially in the early post-traumatic course, the end-points are not always clearly defined. The clinical signs of the patients may not allow accurate evaluation of the clinical condition of the patient. The ideal marker should be able to distinguish between the inflammatory response and infection allowing earlier identification of patients with a specific disease process.

The CRP is probably the most widely used marker in clinical practice at present, despite its inability to distinguish infection from inflammation, its response in proportion to the initial injury, and its inability to predict outcome. Its use persists due to the inability to furnish an acceptable alternative. The search for inflammatory markers initially concentrated on the initiators and promoters of the inflammatory cascade-endotoxin, TNF-α and IL-1.44,45,53,54 These investigations proved to be disappointing. They appear to be transient and most likely it is the response that they initiate which alters outcome. Recent studies have shown promising results in other markers of the inflammatory cascade. From all the available cytokines, IL-6 seems to be the most reliable marker for systemic inflammation, and LBP appears to be an accurate and early marker of infection. The use of these two markers together may offer the ability to detect the onset of SIRS and allow early intervention to prevent MODS, to distinguish between inflammation and infection and to monitor the response to standard and innovative therapies.

The other marker of immune reactivity which appears to have a high sensitivity and specificity is the HLA-DR antigens on human peripheral mononuclear cells. However, because of the laboratory work requiring antibody staining and flow cytometric analysis they have not found great clinical acceptance over the years.

In this article the existing evidence of the most investigated markers of immune reactivity has been evaluated. It appears that at present the ideal marker, which could be assessed easily with the best predictive outcome, is not available. This is not surprising. The idea that single markers can predict the outcome of multiple causes of sepsis and MODS in diverse populations ignores the complexities of the molecular mechanisms behind the inflammatory processes. The multiple-injured population is diverse and has a wide range of underlying and often multiple pathology. Further studies are indicated to provide solid evidence regarding the efficacy of any of the mediators of the inflammatory cascade. Parameters may have to be combined, since no single marker may be able accurately to predict the clinical course and outcome. The completion of the human genome project and the exciting developments anticipated in clinical genetics will promote the introduction of new diagnostic tests and the identification of new candidate genes and will hopefully provide innovative therapies to assist the treatment of the multiple-injured patient.

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INFLAMMATORY SERUM MARKERS IN PATIENTS WITH MULTIPLE TRAUMA

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