Mitochondria and the SIRS Inferno

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MITOCHONDRIA AND THE SIRS INFERNO

INTRODUCTION

There lies within our physiology, an elusive mechanism that appears to govern our response to physical insult and manifests as a syndrome we term the Systemic Inflammatory Response Syndrome (SIRS). Our understanding of this response and the various stimuli that trigger it has become a field of study in its own right. Initially limited by technology, medical science brought together the clinically relevant pieces of this puzzle but fitting them together remained somewhat of a frustrating mystery. How two broadly separate triggers (tissue trauma and infection) could produce an almost identical clinical response which included oedema, ‘leaky capillaries’, and multi-organ dysfunction, appeared to be the key question in SIRS. Much has been hypothesized, but the following discussion hopes to bring to the fore a fresh new paradigm based on studies published in the last year that may finally put the matter to rest.

SIRS in the absence of infection remains sterile/aseptic SIRS and is explained on the basis of triggers (or molecular patterns) that are 'damage' or trauma related i.e Damage Associated Molecular Patterns (DAMPs). Septic SIRS implies a trigger/molecular pattern that is pathogen associated i.e Pathogen Associated Molecular Patterns (PAMPs). These molecular Patterns are Recognised by Receptors in the innate immune system called Pattern Recognition Receptors (PRR’s).

DAMPs have remained far more enigmatic than PAMPs owing to the lack of clarity as to whether the products of cellular debris per se were triggering PRRs, unidentified septic foci, or both. Even in the clear absence of pathogens, sterile SIRS proceeded along a similar pathophysiological path as sepsis.

Now it has come to light that the DAMP trigger may be, what we still contentiously termed, an ‘organelle’ – the mitochondrion. The following discussion highlights the findings by a group of researchers and trauma physicians providing evidence directly linking mitochondrial degradation products (MTD’s) with the SIRS response, and possibly directly to acute lung injury (ALI).

Their findings and associated studies allow us to explore the growing acceptance of the endosymbiotic theory relating to the mitochondrion. This states that the mitochondrion, by virtue of its structure, unique DNA, and independent regenerative capacity, was originally a prokaryotic cell that may have been taken up by a eukaryotic cell line. The innate immune system thus recognises the mitochondria released from traumatised cells as foreign bacteria and the PRR trigger then fires off the cascading inflammatory inferno.
INFLAMMATION AND SIRS

Inflammation

The Inflammatory response, sometimes known as the stress response, was first described by Sir David Cuthbertson then subsequently advanced by Francis Moore and colleagues. They describe a biphasic response to injury consisting of immune, inflammatory and metabolic components.

The first phase, ebb, represents a coordinated response directed towards immediate survival. There is profound peripheral vasoconstriction, hypothermia and shunting of blood and substrate to vital organs. This phase is commonly termed shock. Substrate is mobilized and delivered to vital tissue beds yet at the same time energy expenditure is decreased. Shock represents the activation of responses that, if successful, ensure initial survival following injury. In Cuthbertson’s time this involved a 24 hour period that was intuitively derived. Mechanisms to conserve water and salts as well as translocate blood from the peripheral to the central circulation are activated. In all but the most extreme cases this results in a reversal of the initial stimulus precipitating shock. However, shock is a treatable disease i.e. unanaesthetized shock, where the intervention is fluid administration. However, it also occurs under controlled conditions such as elective surgery (anaesthetized shock).

Anaesthetic induction agents in common use produce a state of profound dilation of the venous capacitance system, decreasing blood return to the heart and thus diminishing cardiac output. This activates the same conservative mechanisms as in the uncontrolled situation. Intervening to expand the circulating volume in either situation stimulates signals indicating that survival appears likely. As a result there is a transition to the second phase of the response, (flow) and known currently as hypermetabolism.

\[ \text{y axis represents any physiologic or metabolic parameter} \]

[Graph showing Ebb and Flow phases]
Systemic Inflammatory Response Syndrome

In 1992, the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) introduced a definition for systemic inflammatory response syndrome (SIRS). The idea behind defining SIRS was to define a clinical response to a non-specific insult of either infectious or non-infectious origin. SIRS is defined as 2 or more of the following variables:

- Fever of more than 38°C or less than 36°C
- Heart rate of more than 90 beats per minute
- Respiratory rate of more than 20 breaths per minute or a PaCO2 level of less than 32 mm Hg
- Abnormal white blood cell count (>12,000/µL or < 4,000/µL or >10% bands)

SIRS is non-specific and can be caused by ischaemia, inflammation, trauma, infection, or a combination of several insults. SIRS is not always related to infection. Infection is defined as "a microbial phenomenon characterized by an inflammatory response to the microorganisms or the invasion of normally sterile tissue by those organisms."

Bacteraemia is the presence of bacteria within the blood stream. This condition does not always lead to SIRS or sepsis. Sepsis is the systemic response to infection and is defined as the presence of SIRS in addition to a documented or presumed infection. Severe sepsis meets the aforementioned criteria and is associated with organ dysfunction, hypo-perfusion, or hypotension.

Sepsis-induced hypotension is defined as "the presence of a systolic blood pressure of less than 90 mm Hg or a reduction of more than 40 mm Hg from baseline in the absence of other causes of hypotension. Patients meet the criteria for septic shock if they have persistent hypotension and perfusion abnormalities despite adequate fluid resuscitation. Multi-Organ Dysfunction or MODS is a state of physiological derangements in which organ function is not capable of maintaining homeostasis.

Although not universally accepted terminology, severe SIRS and SIRS shock are terms that some authors have proposed. These terms suggest organ dysfunction or refractory hypotension related to an ischaemic or inflammatory process rather than to an infectious aetiology.
PATHOPHYSIOLOGY

SIRS, independent of the aetiology, has the same pathophysiologic properties, with minor differences as regards initiating cascades. Many consider the syndrome a self-defence mechanism. Inflammation is the body's response to non-specific insults that arise from chemical, traumatic, or infectious stimuli. The inflammatory cascade is a complex process that involving humoral and cellular responses, complement, and cytokine cascades. Bone best summarized the relationship between these complex interactions and SIRS as the following 3-stage process:

**Stage I:** Following an insult, local cytokine is produced with the goal of inciting an inflammatory response, thereby promoting wound repair and recruitment of the reticular endothelial system.

**Stage II:** Small quantities of local cytokines are released into circulation to improve the local response. This leads to growth factor stimulation and the recruitment of macrophages and platelets. This acute phase response is typically well controlled by a decrease in the proinflammatory mediators and by the release of endogenous antagonists. The goal is homeostasis.

**Stage III:** If homeostasis is not restored, a significant systemic reaction occurs. The cytokine release leads to destruction rather than protection. A consequence of this is the activation of numerous humoral cascades and the activation of the reticular endothelial system and subsequent loss of circulatory integrity. This leads to end-organ dysfunction.

Bone also endorsed a 'multi-hit' theory behind the progression of SIRS to organ dysfunction and possibly MODS. In this theory, the event that initiates the SIRS cascade primes the pump. With each additional event, an altered or exaggerated response occurs, leading to progressive illness. The key to preventing the multiple hits is adequate identification of the cause of SIRS and appropriate resuscitation and therapy.

Trauma, inflammation, or infection leads to the activation of the inflammatory cascade. When SIRS is mediated by an infectious insult, the inflammatory cascade is often initiated by endotoxin or exotoxin. Tissue macrophages, monocytes, mast cells, platelets, and endothelial cells are able to produce a multitude of cytokines. The cytokines tissue necrosis factor-a (TNF-a) and interleukin (IL)–1 are released first and initiate several cascades. The release of IL-1 and TNF-a (or the presence of endotoxin or exotoxin) leads to cleavage of the nuclear factor-kB (NF-kB) inhibitor. Once the inhibitor is removed, NF-kB is able to initiate the production of mRNA, which induces the production other proinflammatory cytokines.

IL-6, IL-8, and interferon gamma are the primary proinflammatory mediators induced by NF-kB. In vitro research suggests that glucocorticoids may function by inhibiting NF-kB. TNF-a and IL-1 have been shown to be released in large
quantities within 1 hour of an insult and have both local and systemic effects. In vitro studies have shown that these 2 cytokines given individually produce no significant hemodynamic response but cause severe lung injury and hypotension when given together. TNF-α and IL-1 are responsible for fever and the release of stress hormones (norepinephrine, vasopressin, activation of the renin-angiotensin-aldosterone system).

Other cytokines, especially IL-6, stimulate the release of acute-phase reactants such as C-reactive protein (CRP) and procalcitonin. Of note, infection has been shown to induce a greater release of TNF-α than trauma, which induces a greater release of IL-6 and IL-8. This is suggested to be the reason higher fever is associated with infection rather than trauma.

The proinflammatory interleukins either function directly on tissue or work via secondary mediators to activate the coagulation cascade, complement cascade, and the release of nitric oxide, platelet-activating factor, prostaglandins, and leukotrienes. Numerous proinflammatory polypeptides are found within the complement cascade.

Protein complements C3a and C5a have been the most studied and are felt to contribute directly to the release of additional cytokines and to cause vasodilatation and increasing vascular permeability. Prostaglandins and leukotrienes incite endothelial damage, leading to multiorgan failure. Polymorphonuclear cells (PMNs) from critically ill patients with SIRS have been shown to be more resistant to activation than PMNs from healthy donors, but, when stimulated, demonstrate an exaggerated microbicidal response. This may represent an autoprotective mechanism in which the PMNs in the already inflamed host may avoid excessive inflammation, thus reducing the risk of further host cell injury and death.
Inflammation and Coagulation

The correlation between inflammation and coagulation is critical to understanding the potential progression of SIRS. IL-1 and TNF-a directly affect endothelial surfaces, leading to the expression of tissue factor. Tissue factor initiates the production of thrombin, thereby promoting coagulation, and is a proinflammatory mediator itself. Fibrinolysis is impaired by IL-1 and TNF-a via production of plasminogen activator inhibitor-1. Proinflammatory cytokines also disrupt the naturally occurring anti-inflammatory mediator's antithrombin and activated protein-C (APC). If unchecked, this coagulation cascade leads to complications of microvascular thrombosis, including organ dysfunction. The complement system also plays a role in the coagulation cascade. Infection-related procoagulant activity is generally more severe than that produced by trauma.

The cumulative effect of this inflammatory cascade is an unbalanced state with inflammation and coagulation dominating. To counteract the acute inflammatory response, the body is equipped to reverse this process via counter inflammatory response syndrome (CARS). IL-4 and IL-10 are cytokines responsible for decreasing the production of TNF-a, IL-1, IL-6, and IL-8. The acute phase response also produces antagonists to TNF-a and IL-1 receptors. These antagonists either bind the cytokine, and thereby inactivate it, or block the receptors. Comorbidities and other factors can influence a patient's ability to respond appropriately. The balance of SIRS and CARS determines a patient's prognosis after an insult. Some researchers believe that, because of CARS, many of the new medications meant to inhibit the proinflammatory mediators may lead to deleterious immnosuppression.
Mitochondria, aside from producing metabolic energy, serve as biosensors for oxidative stress, and eventually become effector organelles for cell death through apoptosis. The extent to which these manifold mitochondrial functions are altered by previously unrecognized actions of traumatic and or anaesthetic agents seems to explain and link a wide variety of perioperative phenomena that are currently of interest to both traumatologists and anaesthesiologists from both a clinical and a scientific perspective.

The Bioenergetic Process

Mitochondria produce the energy needed for normal cellular function and metabolic homeostasis by oxidative phosphorylation, a process conducted by a series of five enzyme complexes located on the inner mitochondrial membrane.

Mitochondrial Biogenesis

The mitochondrion, unique among mammalian organelles, contains multiple copies of a small circular genome of approximately 16,000 nucleotide base pairs. This mitochondrial DNA (mtDNA) has been completely characterized in humans. mtDNA encodes for some key subunits needed for electron transport and oxidative phosphorylation, although the majority of mitochondrial proteins needed for normal bioenergetic function are encoded by nuclear DNA (nDNA) and therefore must be imported into the mitochondrial matrix from the cell cytosol. The expression of the mitochondrial genome itself requires a single mitochondrial transcription factor that arises from the nuclear genome.
Overall, the human mitochondrial genome encodes for 13 peptides (subunits of complexes I, III, and IV and the ATP synthase complex), 2 ribosomal ribonucleic acids (RNAs), and 22 transfer RNAs. Nuclear DNA encodes for at least 1,000 proteins that are needed for mitochondrial bioenergetic and metabolic functions and for mtDNA expression and replication. Although there may be as many as 1,000 copies of mtDNA in most cells, acquired mtDNA point mutations and base pair deletions are extremely rare and are normally found in only a minute proportion of total mtDNA despite the fact that mtDNA, unlike nDNA, lacks histone protection and is surrounded by potentially damaging oxidative influences.

This observation supports the hypothesis that there must be effective molecular repair and disposal mechanisms for damaged mtDNA within mitochondria.
THE NEW EMERGING PARADIGM

The Origin of Mitochondria as Cellular Organelles: Endosymbiotic Theory

In the late nineteenth century, botanist Andreas Schimper first suggested the idea that some organelles evolved from the symbiotic union of two different organisms. As he was observing chloroplast division in green plants, he noticed the resemblance of chloroplasts with free-living cyanobacteria. In the early decades of the twentieth century, another botanist, Konstantin Mereschkowski, gave voice to Schimper's idea by proposing the theory of symbiogenesis, which suggested that chloroplasts originated from symbiotic cyanobacteria. Meanwhile, Ivan Emanuel Wallin proposed that mitochondria arose from bacteria. Their ideas were largely ignored until the 1960s, when the theory finally resurged. By then, scientists had electron microscopes at their disposal to study cells in greater detail.

Scientists could now view cellular organelles and entities that were as small as a few microns. With this revolutionary visual aid, researchers discovered that mitochondria have their own DNA located inside the organelle in the form of circular chromosomes, an observation that was later confirmed by biochemical methods (Nass & Nass 1963; Haslbrunner, Tuppy, & Schatz 1964). In 1967, Lynn Margulis (then Lynn Sagan) gathered diverse microbiological observations to support what is now known as the endosymbiotic theory. Her publication is now a landmark paper on the origin of eukaryotic cells (cells with a nucleus). According to the endosymbiotic theory, mitochondria evolved from ancient symbiotic prokaryotes (organisms without nuclei) that were absorbed into other free-living prokaryotes (Sagan 1967). It took decades for mainstream scientists to accept the hypothesis following evidence and begin calling it a theory.

The Evidence

Mitochondria are self-replicating bodies. Importantly, they are surrounded by two or more membranes, and the innermost of these membranes is very similar in composition to bacteria. Both mitochondria and chloroplast have their own DNA. Mitochondrial DNA has a simple circular structure, which is structurally similar to bacteria DNA and is more or less the same size. Mitochondrial ribosomes, enzymes, and transport systems are all similar to those of bacteria. Moreover, mitochondria are approximately the same size as bacteria. With the advent of molecular biology methodologies, the amount of evidence supporting the endosymbiotic theory has grown. For example, by using genomic sequencing and phylogenetic analysis, scientists have shown that mitochondrial DNA share similar structural motifs with bacterial DNA and that mitochondrial genes originated within proteobacteria (a group of gram-negative bacteria that share common ribosomal RNA sequences). In an exciting recent development, the endosymbiotic theory crossed fields of knowledge to help Trauma and Critical Care physicians tackle a mystery that had been observed for years in the emergency room and postsurgical/trauma intensive care units.
Physicians working in critical care units observe similarities between two different phenomena: systemic inflammatory response syndrome (SIRS) and sepsis. Patients who survive severe trauma or physical injury may develop SIRS resulting in generalized shock and compromised organ function. Sepsis, on the other hand, is a well-characterized phenomenon that occurs during the systemic inflammatory response to severe infection.

Physicians noted that SIRS and sepsis had many of the same symptoms, and both conditions showed clinical similarities. In SIRS, the inflammatory response is triggered by surgery or trauma. In sepsis it is caused by diverse pathogens. It is proposed that the innate immune system recognizes certain molecules or "stimulators" through specialized receptors known as pattern-recognition receptors (PRRs), leading to molecular signalling pathways that result in an inflammatory response (Iwasaki & Medzhitov 2010). What are the "stimulators" in sepsis and in SIRS?

Investigators have discovered that the innate immune system uses PRRs as "microbial sensors" to detect a set of evolutionarily conserved molecules found in a variety of pathogens. These molecules are collectively known as pathogen-associated molecular patterns (PAMPs), and they are expressed in a wide variety of microorganisms, including those that do not cause disease. In patients with severe infections such as sepsis, PAMPs are the major external "stimulators" of the inflammatory response.

In 1994, Polly Matzinger proposed that the immune system does not only respond to pathogens, but it also responds to intracellular alarms that are activated when endogenous molecules are released in the body (Matzinger 1994). In the following years, scientists have experimentally shown that several endogenous molecules are released from damaged tissues, and these molecules were collectively named damage-associated molecular patterns (DAMPs). DAMPs are capable of initiating an inflammatory response similar to that produced by PAMPs (Lotze et al. 2007), even when there are no microbial infections present. So, why are the signalling pathways triggered by PAMPs in sepsis (external "stimulators") and DAMPs in SIRS (internal or endogenous "stimulators") so similar, and do these pathways overlap?
‘Circulating Mitochondrial DAMPs Cause Inflammatory Responses to Injury’

It turns out that our knowledge of mitochondria — that they were evolutionarily derived from bacteria and share conserved structural motifs with prokaryotes — can explain why the signalling pathways activated by external and internal triggers are so similar. With this idea in mind, Qin Zhang and colleagues proposed a new and intriguing hypothesis: They suggested that mitochondrial DNA and proteins may act as DAMPs, triggering the same pathways activated by bacterial PAMPs. This hypothesis would explain the similarities observed between the immune response to infection and the immune response to trauma (Zhang et al. 2010).
Mitochondria and Bacteria Use the Same Mechanisms to Trigger Immune Responses

To gather evidence to test their hypothesis, Zhang and colleagues observed whether mitochondrial DAMPs were released by trauma patients after severe injury. Assuming that mitochondria evolved from bacteria, they looked for two known bacterial PAMPs: DNA and formyl peptides. In one experiment, they measured the release of mitochondrial DNA into the circulation of major trauma patients. As they expected, they found very high levels of mitochondrial DNA in the trauma patients' blood compared with that of control volunteers. They also detected high levels of mitochondrial DNA in bones after fractures were repaired by orthopaedic surgeons. Both of these postinjury measurements confirmed that signature mitochondrial DAMPs are released into the circulation after major bodily injury.

Where do these mitochondria come from? It is very likely that injuries cause cell lysis, degradation, and tissue necrosis, which release the contents of cells, including damaged mitochondria. Because each cell contains many mitochondria and there are many thousands of damaged cells in trauma tissue, severe trauma can result in the release of large amounts of mitochondrial DAMPs into the blood.

In another experiment, Zhang's group analysed how mitochondrial-derived DAMPs activate the immune response. Only bacteria and mitochondria have their proteins N-formylated, therefore, they are the only known sources of N-formyl peptides in nature. Formyl peptides can attract neutrophils (essential to the innate immune system). They can also activate neutrophils by specifically binding to the formyl peptide receptor-1 (FPR1), which is found on the surface of these cells.
The activation of neutrophils promotes the inflammatory response by releasing chemical mediators and activating several enzymes known as MAP kinases. Using mitochondrial-derived DAMPs, they were able to activate FPR1 and MAP kinases, which confirmed the presence of formyl peptides in mitochondrial DAMPs. Mitochondrial DNA can also bind to neutrophils through a specific receptor called the toll-like receptor 9 (TLR9) located on their surfaces. TLR9 is a member of the PRRs. By binding to TLR9, mitochondrial DNA can also activate the MAP kinases. Zhang's group concluded that the immune response to injury "mimics sepsis" because mitochondrial DAMPs activate neutrophils through PRRs and FPR1, which normally would be activated by bacterial PAMPs.

A highly interesting question Zhang and his colleagues asked was if circulating mitochondrial DAMPs could cause neutrophil-mediated organ injury. To answer this question, they intravenously injected mitochondrial DAMPs into rats to see if they could produce organ injury in vivo. After exposure to the DAMPs, they found that mitochondrial DAMPs produced systemic inflammation in several tissues, including a "marked-inflammatory lung injury", which is a major cause of ALI/ARDS in critically ill patients. In contrast, the control rats showed no evidence of inflammation.

In scientific research, a new discovery often opens the door to even more questions. Are there additional DAMPs associated with mitochondrial components that can trigger an immune response to trauma? Does the quantity of mitochondrial DAMPs released after trauma determine a patient's clinical outcome? In addition, can high amounts of circulating DAMPs be used as a marker to predict the severity of the inflammatory response and mortality?
SEPTIC AND STERILE SIRS (CLINICAL ASPECTS)

Definitions (ACCP/SCCM)

**Sepsis:**
- Known or suspected infection, plus
- >2 SIRS Criteria.

**Severe Sepsis:**
- Sepsis plus >1 organ dysfunction.
- MODS.
- Septic shock

**Septic Shock**
- Sepsis-induced with hypotension despite adequate resuscitation along with the presence of perfusion abnormalities which may include, but are not limited to lactic acidosis, oliguria, or an acute alteration in mental status.

**Sterile (Non-Septic SIRS)**

Non-infectious causes of SIRS include:
- trauma, burns, pancreatitis, ischaemia, and haemorrhage.
- Complications of surgery
- Adrenal insufficiency
- Pulmonary embolism
- Complicated aortic aneurysm
- Cardiac tamponade
- Anaphylaxis
- Drug overdose

**Laboratory Studies**

In order to completely evaluate for SIRS, a full blood cell count with differential to evaluate for leucocytosis or leucopenia is required. Routine screenings may also include a basic metabolic profile. Other laboratory tests should be individualized based on the patient history and physical examination findings.

A significant amount of research has evaluated the use of acute-phase reactants to help differentiate infectious from non-infectious causes of SIRS. Arkader et al compared procalcitonin (PCT) with CRP in their ability to differentiate infectious from non-infectious causes. Their observational prospective study in a paediatric ICU showed that PCT was able to differentiate between infectious and non-infectious SIRS, while CRP was not. Another study confirmed that PCT is a better
indicator of early septic complications than CRP in complex populations such as trauma patients.

Caution must be used in interpreting PCT results in elderly patients. Lai et al demonstrated that PCT is useful in predicting bacteraemia in elderly patients but was not an independent marker for local infections.

PCT is becoming increasingly available to physicians as a point-of-care test. Currently, availability of this assay will vary by medical centre.

Selberg et al reviewed PCT and CRP, in addition to looking at IL-6 and C3a. Their research showed that PCT, IL-6, and C3a were again more reliable in distinguishing infectious from non-infectious causes.

Patients who meet SIRS criteria and have increased IL-6 levels (>300 pg/mL) have been shown to be at increased risk for complications such as pneumonia, MODS, and death.

Leptin, a hormone generated by adipocytes that acts centrally on the hypothalamus to regulate body weight and energy expenditure, is an emerging marker that correlates well with serum IL-6 and TNF-alpha levels. Using serum leptin levels with a cut-off of 38 mug/L, researchers have been able to differentiate sepsis from non-infectious SIRS with a sensitivity of 91.2% and a specificity of 85%. This test is not yet readily available for clinical practice in the USA.

Other Tests

Blood cultures, urinalysis and culture, cardiac enzymes, amylase, lipase spinal fluid, and liver profiles are among the numerous laboratory tests to consider.

Blood lactate assessments are often performed in critically ill patients. These are felt to be indicators of anaerobic metabolism associated with tissue dysoxia. Levels are commonly elevated from increased peripheral intraorgan production and reduced hepatic uptake and reduced renal elimination. Based on numerous studies, lactate levels correlate strongly with mortality.
The current SIRS paradigm

The patient is initially injured in some way. The result of this injury is activation of host defence mechanisms, including release of inflammatory cytokines, particularly interleukin-6 and tumour necrosis factor alpha.

The physiologic manifestations of this process include tachypnoea, tachycardia, leukocytosis and pyrexia, and we call this the systemic inflammatory response. The body is, in effect, responding to the source of inflammation and making physiologic compensation for the systemic upset.

If the patient is unable to adequately compensate, and suffers acute organ failure, then he/she requires critical care interventions – usually mechanical ventilation, often with cardiovascular support.

The patient has undergone a “first hit”. He/she is now vulnerable to further injury. At this point one of three things may happen: 1) commencement of resolution, 2) the injury and inflammatory and or inflammatory response may persist. 3) The patient may develop a second (or third or fourth) injury, such as nosocomial pneumonia, ventilator induced lung injury or bacterial translocation from the gut, which stokes up the inflammatory response.

Persistent inflammation leads to widespread endothelial dysfunction, and ischaemic tissue injury (due to hypotension, intravascular thrombosis, tissue oedema, abnormal oxygen extraction).

The result of this is sequential organ damage – multi-organ dysfunction. Examples of this are an increase in the alveolar to arterial oxygen gradient, a reduced ejection fraction, agitation or coma, a reduction in creatinine clearance, and increase in serum bilirubin, a decrease in platelets and clotting factors etc.

As the disease process progresses, multi-organ dysfunction becomes multi-organ failure. This is characterized by the requirement for external interventions to maintain homeostasis – mechanical ventilation, inotropes and vasopressors, renal replacement therapy, continuing blood product transfusions etc.

The patient becomes severely catabolic, physiologic reserve deteriorates and neuroendocrine exhaustion occurs. The latter is characterized by the inability of the patient to mount an appropriate endocrine response to ongoing stress and inflammation.

The majority of patients who develop multi organ failure succumb, due to inability to wean external interventions (usually mechanical ventilation and vasopressors). Death is inevitably as a result of withdrawal of this support.
CLINICAL MANAGEMENT OF SIRS

The initial medical care should include prompt initiation of pertinent laboratory testing and imaging studies after obtaining a history and performing a physical examination. Treatment should then be focused based on possible inciting causes of systemic inflammatory response syndrome (SIRS; e.g. appropriate treatment of acute myocardial infarction differs from the treatment of community-acquired pneumonia or pancreatitis).

Empiric antibiotics are not indicated for all patients with SIRS. Indications for antibiotic therapy include suspected or diagnosed infectious aetiology (e.g., urinary tract infection, pneumonia, cellulitis), hemodynamic instability, neutropaenia (or other immunocompromised states), and asplenia (due to the potential for overwhelming postsplenectomy infection [OPSI]). When feasible, culture data should always be obtained prior to initiating antibiotic therapy. Empiric antibiotic therapy should be guided by available practice guidelines and knowledge of the local antibiogram, as well as the patient's risk factors for resistant pathogens and allergies. Once a bacteriologic diagnosis is obtained, narrowing the antibiotic spectrum to the most appropriate therapy is critical.

Because of increasing bacterial resistance, broad-spectrum antibiotics should be initiated when an infectious cause for SIRS is a concern but no specific infection is diagnosed. With the increasing prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in the community, vancomycin or another anti-MRSA therapy should be considered.

Gram-negative coverage with cefepime, piperacillin-tazobactam, carbapenem (imipenem, meropenem, or doripenem), or a quinolone is reasonable.

Recent exposure to antibiotics (typically within 3 months) must be considered when choosing empiric regimens because recent antibiotic therapy increases the risk for resistant pathogens. Care must be made not to use an antibiotic to which the patient is allergic. This may be a second hit and lead to worsening SIRS. Because of the high prevalence of patients with penicillin allergy, a quinolone or aztreonam is reasonable alternatives for gram-negative coverage.

Antiviral therapy has no role in SIRS unless the patient is immunocompromised or the patients presents for evaluation during influenza season.

Empiric antifungal therapy (fluconazole or an echinocandin) can be considered in patients who have already been treated with antibiotics, patients who are neutropaenic, patients who are receiving total parenteral nutrition (TPN), or patients who have central venous access in place.

Although empiric antibiotics may be reasonable in many situations, the key is to stop antibiotics when infection is ruled out or narrow the antibiotic spectrum once
a pathogen is found. Proper culture data must be obtained prior to any antibiotic therapy. Antibiotics prior to culturing a patient may be a cause of sterile sepsis.

TNF-a and IL-1 receptor antagonists, antibradykinin, platelet-activating factor receptor antagonists, and anticoagulants (antithrombin III) have been studied without showing statistically significant benefits in SIRS (with variable results for sepsis and septic shock). These medications have no role in treating patients who meet criteria for SIRS only.

Drotrecogin alpha, a recombinant form of APC, warrants further comment. APC reduces microvascular dysfunction by reducing inflammation and coagulation and increasing fibrinolysis.

The Patients in the Recombinant Human Activated Protein-C Worldwide Evaluation in Severe Sepsis (PROWESS) study demonstrated its ability to reduce 28-day all-cause mortality following severe sepsis. Further studies have demonstrated that it is best used in patients with gram-negative septic shock. In the PROWESS study, no clinical benefit was found in patients with acute physiology and chronic health evaluation (APACHE) scores of less than 25, and further studies have demonstrated worse outcomes in patients with lower APACHE scores.

Therefore, APC has no role in most SIRS cases unless the clinical presentation is consistent with septic shock. APC has strict inclusion and exclusion criteria that must be considered in all patients prior to initiating therapy. The greatest benefit of APC has been demonstrated when this medication is initiated early in the inflammatory cascade.

Steroids for sepsis and septic shock have been extensively studied, but no SIRS-specific studies have been performed to date.

The initial research in sepsis and septic shock showed a trend toward worse outcomes when treating with high doses of steroids (methylprednisolone sodium succinate 30 mg/kg every 6 h for 4 doses) compared with placebo.

However, research into low-dose steroids (200-300 mg of hydrocortisone for 5-7 d) improved survival and the reversal of shock in vasopressor-dependent patients. As mentioned above, the inflammatory mediators and receptors associated with infectious insults (i.e., septic shock) are the same as those of non-infectious insults (i.e., trauma, inflammatory conditions, and ischaemia). Low-dose steroids should be considered on an individual basis for patients with refractory hypotension (i.e., septic shock) despite adequate fluid resuscitation and appropriate vasopressor administration. Prior to initiating steroid therapy, physicians must consider the potential risk of steroids (such as stress ulcers and hyperglycemia).
Current data do not support using ACTH stimulation testing to determine patients who should receive steroid therapy. Patients receiving steroids require careful monitoring for hyperglycemia.

Patients who are hypotensive should receive intravenous fluids, and, if still hypotensive after adequate resuscitation, vasopressor agents should be administered while carefully monitoring hemodynamic status. All patients should have adequate intravenous access and commonly require 2 large-bore intravenous lines or a central venous catheter.

Hyperglycemia, a common laboratory finding in SIRS, even in individuals without diabetes, has numerous deleterious systemic effects.

An increase of counterregulatory hormones, namely cortisol and epinephrine, and relative hypoinsulinemia lead to increased hepatic glucose production, increased peripheral insulin resistance, and increased circulating free fatty acids. This has direct inhibitory action on the immune system. Oxidative stress and endothelial cell dysfunction, along with proinflammatory cytokines (IL-6, IL-8, TNF-a) and other secondary mediators (NF-kB) have all been implicated as causes of cellular injury, tissue damage, and organ dysfunction in patients with hyperglycemia.

Intensive control of blood glucose levels has been shown to diminish in-hospital morbidity and mortality in both the surgical and medical intensive care setting.

Supplemental oxygen should be provided to any patient that demonstrates an increased oxygen requirement or decreased oxygen availability. Oxygen can be provided via nasal cannula or mask, or, in certain situations, ventilator support may be required to maximize oxygen delivery. Supplying supraphysiologic oxygen has shown mixed results in a multitude of studies. Providing too much oxygen in a patient with severe chronic obstructive pulmonary disease (COPD) should be avoided because it can depress their respiratory drive. Patients who do not respond to increased oxygen supply have a poor prognosis. Patients with associated respiratory failure who require mechanical ventilation should be treated with low tidal volume mechanical ventilation (6 mL/kg).

**Surgical Care**

In general abscesses or drainable foci of infection (e.g. haematomas) should be drained expeditiously. Patients with acute surgical issues (e.g., ruptured appendix, cholecystitis) that cause SIRS should be treated with appropriate surgical measures. Prosthetic devices should be removed in a timely manner, when clinically feasible.
Zhang et al. found that, like bacterial DNA released following sepsis, mitochondrial DNA released by severe trauma can also act through toll-like receptor-9 (TLR9) to activate neutrophils through activation of p38 MAP kinase (MAPK) enzyme. Similarly, formylated peptides released from bacteria and mitochondria in these settings attract neutrophils by the process of chemotaxis to sites of inflammation and injury through formyl peptide receptor-1 (FPR1). In both cases, the outcome may be acute lung injury, as part of the systemic inflammatory response syndrome (SIRS).

Calfee, Matthay, Nature March 2010
DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS

Future Diagnostic tools

Mitochondria bear bacterial molecular motifs including bacterial-style CpG-DNA and formylated peptides that act upon human innate immune cells via Toll-Like Receptor-9 (TLR-9) and G-protein coupled formyl peptide receptors. Major injury releases mitochondrial detritus with mitochondrial DNA (mtDNA) reaching levels in the circulation that activate immune cells.

Mitochondrial peptides and mtDNA signal through Formyl Peptide Receptor-1 and TLR9 respectively, promoting neutrophil (PMN) cytosolic calcium ([Ca2+]i) release and entry as well as causing phosphorylation of P38- and P44/42-mitogen activated protein kinases (MAPK). These signals cause PMN migration to and degranulation in the lung and liver, initiating PMN-mediated oxidative organ injury there.

Thus cellular disruption by major trauma releases mitochondrial DAMPs into the circulation that have evolutionarily conserved molecular signatures similar to bacterial PAMPs. Release of such mitochondrial 'enemies within' locally is likely a necessary step in the initiation of inflammation, which then leads to wound healing. In overwhelming injuries however, the same pathways become activated systemically. In that case, widespread activation of innate immunity leads to a SIRS syndrome that is similar to sepsis and has a high lethality even with modern care. Thus innate immune activation is likely to be beneficial at the local level in the case of minor, survivable injuries. But conversely, global innate immune activation appears to promote early organ failure and death in the case of major injuries that would not have been survivable early in the human evolutionary process.

Trauma surgeons who support these patients now with intensive care must accept the SIRS response as a consequence of survival. But for early Man, it is likely that in the case of major injuries selection pressures at the societal level would have favoured early death over prolonged illnesses that could have immobilized the entire social group or exhausted its resources.

Is the new diagnostic way forward Mitochondrial Degradation Product markers?

MTD biomarkers

SIRS can be caused by either a non-infectious event such as direct trauma, ischaemia or toxins or it can result from an invasive microbial infection, in which case the condition is termed sepsis. It is often difficult to distinguish between these two conditions and the treatment of each is vastly different.
Sepsis requires antibiotic therapy whereas anti-microbials are not helpful in non-infective SIRS. In fact, despite the enormous effort and costs devoted to antibiotic therapy, they often lead to the emergence of resistant infections and worse outcomes. Conversely, anti-inflammatory therapies often fail to improve and can increase mortality in early sepsis whereas they might improve outcomes if patients with non-infective SIRS could be reliably identified.

A new test - a PCR based assay of circulating MTD’s – is currently being patented, to distinguished sepsis from non-infective SIRS directly. The assay measures the relative amounts of circulating mitochondrial and bacterial DNA where elevated levels of mitochondrial DNA specific for tissue injury are an indicator of non-infective SIRS and elevated levels of bacterial DNA indicate sepsis.

![Bar chart showing Quantitative PCR (qPCR) for Cyt B DNA in plasma from trauma patients (µg/mL)](chart)

BIDMC 1363: Diagnostic Test to Distinguish Sepsis from a Non-infective Systemic Inflammatory Response (SIRS)

- Rapid, PCR-based assay of circulating bacterial and mitochondrial DNA
SUMMARY

Intensive care units are increasingly burdened by inflammatory complications related to illness or injury. Systemic Inflammatory Response Syndrome (SIRS) is a serious consequence of traumatic injury. The SIRS inflammatory response was once believed to result from gut bacterial translocation into the systemic circulation via the portal vein. Research in the 1990s disproved this hypothesis. Now, recent evidence following studies by trauma surgeons and immunologist at Harvard Medical School in Boston, may have revealed the cause of SIRS.

Hauser, Zhang and colleagues found that the plasma of traumatically injured patients contained 1,000 times more mitochondrial DNA than is found in normal plasma. Hauser hypothesized that mitochondrial debris, released from traumatically injured cells and perceived by the body as invading microorganisms, initiates an inflammatory response and SIRS.

They further explored this connection by injecting mitochondrial DNA into the lung tissues of experimental rats. Neutrophils migrated to the area where the mitochondrial DNA was injected and an inflammatory response resulted. When bacteria are responsible for inflammation, antibiotics are effectively used for treatment. However, in SIRS antibiotics are not always helpful. Having identified mitochondrial DNA fragments as contributing to the inflammatory response in traumatic injuries, pharmacological intervention to block or slow the inflammatory response may be on the horizon. Identification of this new mechanism that initiates inflammation may lead to effective treatment for a syndrome previously difficult to understand and to treat.
REFERENCES