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THE PATHOPHYSIOLOGY OF SEPTIC SHOCK

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ABSTRACT

While writing this review, Microbiologists, in collaboration with Anaesthetists and Intensivists, have kept in mind the incidence of sepsis and septic shock along with its mortality and morbidity. Moreover, recent advances have been made in the in understanding of the pathophysiology of sepsis and the genesis of septic shock. This has led to the development of new approaches to treatment. Hence early identification and aggressive management will finally lead to reduction of overall mortality. Thus we have tried to highlight the evolving concepts underlying the pathogenesis of septic shock for better understanding and appropriate management outcomes.

KEY WORDS

Septic Shock, Pathophysiology, Sepsis.

INTRODUCTION:

"… the microorganisms that seem to have it in for us …. turn out …. to be rather more like bystanders … It is our response to their presence that makes the disease. Our arsenals for fighting off bacteria are so powerful … that we are more in danger from them than the invaders[1].We live in a world where the microbes are always trying to get at us, to tear us cell from cell, and we only stay alive and whole through diligence and fear"[1].As profoundly expressed by Lewis Thomas justifies the need for upgrading the current knowledge on sepsis and its defense mechanisms.

The normal response to microbial infection is a complex inflammatory process that attempts to localize the infection and repair the tissue. This response involves the activation of circulating and fixed phagocytic cells, and the generation of both pro-inflammatory and anti-inflammatory

mediators. A balance between these mediators helps to facilitate tissue healing. Sepsis results when the balance is lost; the inflammatory response extends beyond the infected tissues, and becomes generalized. The process to control infection then becomes uncontrolled and unregulated.

Clinicians, Intensivists and Infectious Disease specialists have all employed different terminologies at this stage for similar but overlapping clinical conditions. So it is best, that we first define these terminologies. In 1992, the American College of Chest Physicians and Society for Critical Care Medicine Consensus Conference Committee, agreed upon the definitions as in Table 1[2].

Table 1

American College of Chest Physicians and Society for Critical Care Medicine Consensus Conference Committee definitions of stages of Sepsis.

Stage	Characteristics
I-**SIRS***	≥2 of following: Temperature>38^0C or <36^0C Heart rate >90/minutes Respiratory rate >20/minute or $PaCO_2$ <32mmHg WBC>12,000/μL or <4000/μL or >10%bands
II-**Sepsis**	SIRS+ concrete evidence of infection
III-**Severe sepsis**	Sepsis + acute associated organ dysfunction / hypotension / hypo perfusion (lactic acidosis, oliguria, acute alteration in mental status)
IV-**Septic Shock**	Sepsis + hypotension (arterial BP‡<90mm Hg systolic or 40mmHg< patient's normal BP)despite adequate fluid resuscitation + perfusion abnormalities (need DA§ >5μg/kg/min or NE / EP <0.25μg/kg)
V-**Refractory Septic Shock**	Septic Shock lasting >1hour and not responding to fluid or pressor agents (DA>15μg/kg/min or NE‖ / EP¶>0.25μg/kg
VI-**MODS**†	Dysfunction of >1 organ requiring intervention to maintain homeostasis *1st degree MODS* – due to direct result of well defined insult, organ dysfunction occurs early *2nd degree MODS* – due to host response.

Parenthesis:

* : *Systemic inflammatory response syndrome*
† : *Multi organ dysfunction syndrome*
‡ : *blood pressure*
§ : *Dopamine*
‖ : *Norepinephrine*
¶ : *Epinephrine*

Fever, hypothermia, tachypnoea and tachycardia – often herald the onset of sepsis, i.e., the systemic inflammatory response to microbial invasion. When counter regulatory mechanisms are overwhelmed and the microbe moves from a local site to the blood stream, homeostasis may fail and dysfunction of major organs supervene leading to severe sepsis. Further failure of the counter regulatory mechanisms characterized by hypotension leads to septic shock and finally to multi organ dysfunction syndrome (MODS). So sepsis, severe sepsis and septic shock all represent the increasing severe stages of the same disease or the increasing severity of an individual's systemic response to infection. Figure 1 illustrates the inter relation between SIRS, infection and sepsis. Hence, as sepsis progresses to septic shock, the risk of dying increases substantially.

Laboratory criteria of Sepsis:

Abnormalities which occur early in septic response:

- Leukocytosis with a left shift
- Thrombocytopenia
- Hyperbilirubinemia
- Proteinuria
- Leucopenia (toxic granules)
- Respiratory alkalosis

As the septic response becomes more severe

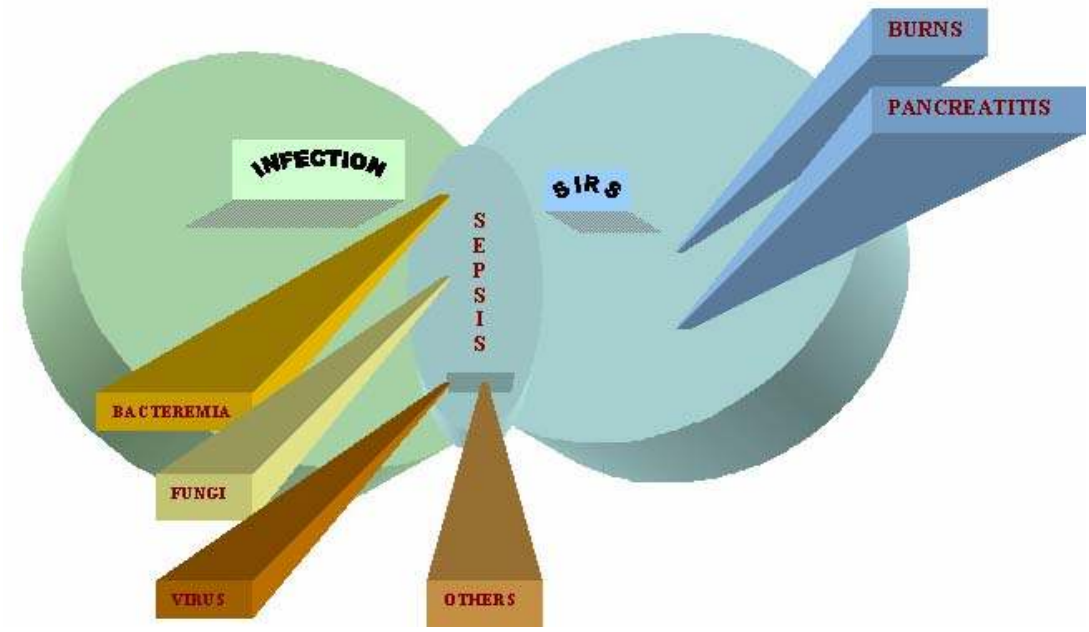
- Thrombocytopenia *worsens* (prolongation of thrombin time, ↓fibrinogen)
- Azotemia
- Hyperbilirubinemia *worsens*
- aminotransferases
- Microangiopathic changes on blood smear → DIC
- Metabolic alkalosis
- Decreased Serum albumin
- Hyperlipedemia
- Arterial Blood Gas Analysis→hypoxemia, initially correctable, later refractory to 100% oxygen.

  Chest X-ray – normal / pneumonia / volume over load / diffuse infiltrates of ARDS

  ECG – sinus tachycardia / non specific ST-T wave abnormalities.

Figure 1

The inter relation between SIRS, infection and sepsis



EPIDEMIOLOGY

Sepsis and Septic Shock are common problems encountered in the ICU. Infact, septic shock with MODS is the leading cause of death in non-coronary ICU's. A prospective observational study[3] demonstrated that, among hospitalized patients meeting the criteria of SIRS, approximately 26% progress to sepsis, 18% to severe sepsis and up to 4% progress to septic shock. The interval from SIRS to severe sepsis and septic shock inversely correlates with the number of SIRS criteria met. Mortality rates in case of sepsis are 16%, which increases to 20% in severe sepsis and further up to 46% in case of septic shock. Mortality rates in children are less (10%) but increases nearly four times (38.4%) when the age group is ≥85years; and the same in case of males is slightly higher (29.3%) as compared to females (27.95). The yearly incidence of sepsis is 50-95 cases per 100,000 populations, and has been increasing by 9% each year[4]. Sepsis accounts for 2% of hospital admissions, however two thirds of all cases of sepsis occur in patients who have been

hospitalized for other illnesses[5]; septic shock, accounts for 10% of admissions to intensive care units[6]. In Guru Teg Bahadur Hospital, a tertiary care facility in East Delhi, having a 12 bedded ICU, it was seen that in a seven month period, 27% of the admissions in ICU were due to severe sepsis leading to MODS. Whereas, in Swamy Dayanand Hospital, a secondary care hospital, with a 5 bedded ICU having state of the art facility, also catering to East Delhi, had 38% admissions in ICU due to sepsis in 2006, which increased to 42% in 2007. More than one million people in the world and 20,000 people in the United States die each year due to septic shock[7]. It is the 13th leading cause of death in the United States[8]. A study estimated 751,000 cases of severe sepsis occur annually in the United States[9]. When patients with HIV are excluded, the incidence of sepsis in men and women is similar. The incidence of sepsis is further expected to rise during the next decade owing to the aging population, a growing immunosuppressed population, the increased use of invasive catheters and prosthetic devices and the growing problem of antimicrobial resistance. In the year 2010, it is estimated that there will be 934,000 new sepsis cases in the United States, and in 2020, the figure will rise to 1,100,000.[9] As regards to the number of patients admitted with sepsis and septic shock, it would be interesting to audit the outcome of these patients by the hospital infection control team or even externally by infection control society of India.

ETIOLOGY

Sepsis can be a response to any class of microorganism. Microbial invasion of the blood stream is not essential for the development of sepsis. Local or systemic spread of microbial signal molecules or toxins can also elicit a response. The occurrence of gram-negative sepsis has diminished over the years to 25-30% in 2000. Gram-positives account for the majority of cases upto 30-50%. In 11-19% cases, the etiology is polymicrobial in nature. Fungi, Viruses and parasites account for 1-4%, but their frequency could be underestimated.[6, 10]. Lastly cultures may be negative in 30% of cases, mainly in patients with community-acquired sepsis who are treated with antibiotics before admission. Figure 2.

Figure 2
Etiology of Sepsis

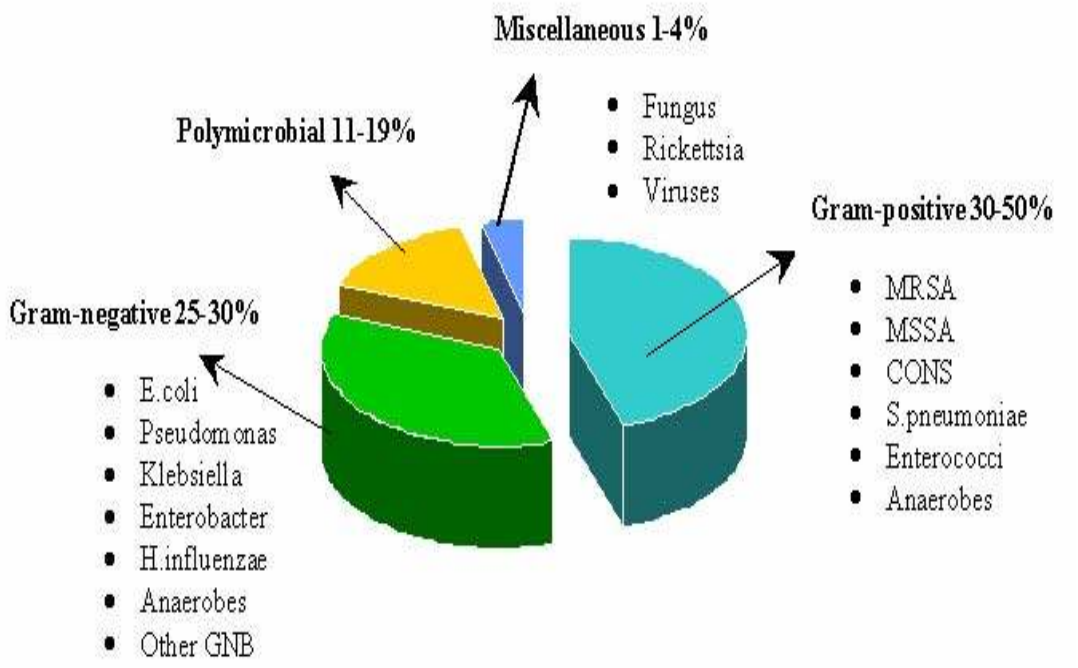


Table 2
Pathogens of Septic Shock in special circumstances

Circumstance	Pathogens
Splenectomy	*S.pneumoniae, H.influenzae, N.meningitides*
Neutropenia (<500/μl)	*P.aeruginosa, S.aureus*, Candida spp
Hypergammaglobulinemia	*S.pneumoniae, P.aeruginosa, E.coli*, MRSA*
AIDS	*P.aeruginosa* (± neutropenia), *P.jerovecii*
Intravascular devices	*S.aureus*, CONS†
Nosocomial infection	MRSA, VRE‡, Candida, MDR GNB§

**: methicillin resistant Staphylococcus aureus*
†: coagulase negative staphylococcus
‡: vancomycin resistant enterococcus
§: gram negative bacilli

The source of infection is usually endogenous. Infections of the chest, abdomen, genitourinary system and primary blood stream cause more than 80% of sepsis [4,6,9]. Rates of pneumonia, bacteremia and multiple site infection have steadily increased over time, whereas abdominal infections have remained unchanged and genitourinary infections have decreased [4,6]. Rarely, the source may even be exogenous. Hemorrhagic and bullous lesion in a septic patient who has recently eaten raw oysters suggests *Vibrio vulnificus* septicemia, while such a lesion in a patient who has recently suffered from a dog bite may indicate blood stream infection due to *Capnocytophaga canimorsus* or *C.cynodegmi*. A cutaneous lesion, consisting bulla surrounded by edema that undergoes central hemorrhage and necrosis, seen almost exclusively in neutropenic patients is ecthyma gangrenosum, usually caused by *Pseudomonas aeruginosa*. Site of infection is an important factor in predicting clinical outcome, e.g. severe sepsis is more likely to occur in nosocomial pneumonia than in bacteremia acquired from indwelling urinary catheter.

Factors that can predispose people to septic shock include [5,6,9] :

- diabetes mellitus
- lymphoproliferative disorders
- cirrhosis
- invasive procedures or devices
- burns
- I/V Drug User
- chronic organ failure
- prolonged antibiotic therapy
- cancer
- Neutropenia

Certain genetic factors have also been implicated e.g. being male, non-white ethnic origin in North Americans and polymorphisms in genes that regulate immunity –LPS binding proteins, bactericidal permeability increasing proteins (BPI), heat shock proteins.[11,12,13]. A septic shock gene has been identified called *Tlr4* gene – mutations in this gene permit bacteria to grow unchecked and when the immune system reacts, it does so violently and release large amount of immune substances into the blood stream. This violent reaction can lead to shock, formation of blood clots and spontaneous bleeding even though the blood is clotting[7].

PATHOPHYSIOLOGY

PAMP and their receptors: The pathophysiology of sepsis is initiated by the pathogen associated molecular patterns (PAMPs) and their recognition by the PAMP receptors or the pattern recognition receptors[14]. The PAMPs include lipopolysaccharide (LPS) of gram-negative bacteria, peptidoglycan and lipoteichoic acid of gram-positive bacteria, fungal glucan, outer membrane protein (OMP), flagellin, fimbriae, heat shock protein (HSP) etc[14]. LPS is the most common and potent of the PAMPs that can potentiate sepsis. The Lipid A region is the toxic portion of this PAMP. It binds to CD_{14} receptor, which is a 55KD glycoprotein expressed on the surface of macrophage, monocytes and neutrophils. CD_{14} is considered a pattern recognition receptor that evolved because of the need for an immediate response to invasion. Although it has a wide

spectrum of binding capacity, but LPS binding has greater affinity among other molecules. The binding of LPS is catalyzed by LPS binding protein. This LPS binding protein is present in approximately 5μg/ml in normal individuals. However in sepsis, its level may be elevated to >100μg/ml. But CD_{14} receptors are not responsible for signal transduction. The signal transducing receptors are the toll like receptors (TLRs). TLRs have extra cellular leucine rich repeat domains and play a critical role in early innate immunity to evading pathogens by sensing microorganisms[14]. Currently there are ten TLRs. TLR-2 interact with peptidoglycan, lipoprotein, lipoteichoic acid and fungal glucan. TLR-4 interacts with LPS and HSP. TLR-5 interacts with flagellin. Other signal transducing receptors are the Nod 1 and Nod 2 receptors [14].

Immunological alterations in septic shock:

Signal transducing pathway: Signal transduction after interaction between PAMP and the TLRs results in activation of numerous adaptors eventually leading to the production of the proinflammatory cytokines like tumor necrosis factor alpha (TNF∝) and interleukin1 (IL-1β).[15] Blood levels of these cytokines are high in most patients with severe sepsis or septic shock[3]. These cytokines have a direct toxic effect on the tissues. They activate phospholipase A_2 and this result in production of arachidonic acid and platelet activating factor (PAF). Arachidonic acid produces prostaglandins and thromboxanes via the cycloxygenase pathway. Prostaglandins are peripheral vasodilators while PAF have a vasoconstrictor and platelet-aggregating function.[3] Administration of cycloxygenase inhibitor Ibuprofen for 48hrs in severe sepsis can suppress production of these metabolites and block this pathway.[3] PAF also stimulate neutrophil aggregation and degranulation as well as promote platelet aggregation[3]. Specific TNF∝ antagonists can abrogate the septic responses and prevent death of experimental animals challenged with endotoxin[3].

Various investigations reports support this logic that persistent increase in circulating TNF∝, IL-6 or IL-8 levels often lead to multiple organ failure and non survival [16]. However, Cavaillon et al likened circulating cytokine concentrations as the "tip of the ice-berg" with respect to physiological responses and therefore may reflect in totality the critical condition of these [16]

Two MoAb (monoclonal antibodies) directed at core LPS and Lipid A have been developed: E5, a murine Monoclonal IgM and HA-1A, a human MoAb[17]. Following encouraging animal studies [18,19], there have been several large prospective, randomized, placebo-controlled clinical trials recently of both E5 and HA-1A. Although improved survival was seen with earlier E5 trials, this finding could not be confirmed in subsequent trials[20] but reduced incidence of organ failure, suggested by previous studies is being confirmed by subsequent trials. Similarly, studies with HA-1A[21] indicated a 28 day reduction in mortality and hence were largely responsible for the clinical approval of treatment in Europe. However the CHESS trial[22] conducted by the US Food and Drug Administration observed a failure to reduce 28day mortality in patients with gram negative infection and septic shock.

Another anti-endotoxin approach is the possibility of using the naturally occurring molecule known as bactericidal permeability increasing protein (BPI) which binds strongly with Lipid A to inhibit the toxicity of endotoxin[16]. Recombinant BPI has been shown to protect against lethal doses of endotoxin in mice and rats[23].In one clinical trial this protein decreased mortality and morbidity among children with fulminant meningococcal meningococcemia[3]. Other studies in humans are being planned. Probably, it may eventually be possible to protect against lipid A by prophylaxis. It has been found that some types of natural lipid A and synthetic lipid A analogues have low or even no toxicity. This has led to the development of lipid A analogues that can block the toxic effects of endotoxin [24].

Up-regulation of stimulatory molecules:

There are several reports suggesting both the beneficial and deleterious effects of the stimulatory molecule – nitric oxide (NO). It is known that macrophages not only recognize microbial products, but in response secrete many mediators like IL-1, IL-6, IL-8, IL-10, IL-12, granulocyte macrophage colony stimulating factor (GMCSF), TNF∝, PAF and leukotrienes in addition to reactive oxygen intermediates. A number of therapeutic options are also available which are associated with blocking of oxygen free

radicals – non-enzymatic scavengers e.g. vitamin C, N- acetyl cysteine etc demonstrate protective effects [25]. There is also up regulation of the respiratory burst by induction of the inducible nitric oxide synthetase (iNOS) leading to relaxation of the tone of arterial smooth muscle by the production of nitric oxide (NO). NO has also been implicated in myocardial depression and is thought to have a direct cytotoxic effects leading to tissue injury and organ failure[26] (Figure 3). There is a possibility that NO synthesis inhibitors may increase survival rates in septic shock by increasing mean arterial pressure and reducing cytotoxic damage[17]. Experimental data are however controversial. In recent animal studies, using N-ω amino-L-arginine (L-NAA) and monomethyl L-arginine (L-NMMA), both NO synthase inhibitor, it has been seen that arterial pressure was increased at the cost of decreasing survival time, increased lactic acidosis and hepatic toxicity at higher doses [27,28]. Evidence, therefore, suggests that, NO inhibition may have both beneficial and deleterious effects depending on both the dosage and the timing of administration[29]. There have been preliminary reports on the administration of L-NMMA in human beings, [30] which suggested that a blockade of NO synthase may increase blood pressure but not cardiac output. Studies looking at morbidity and mortality are under way. Hence, in addition to the dosage, the timing of administration plays an important role.

Relationship between TNFα and NO

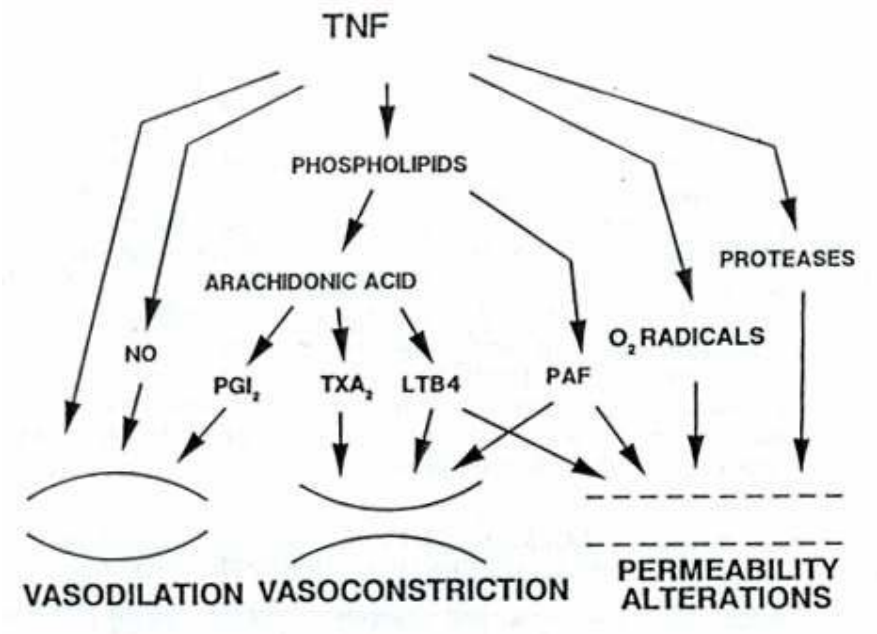
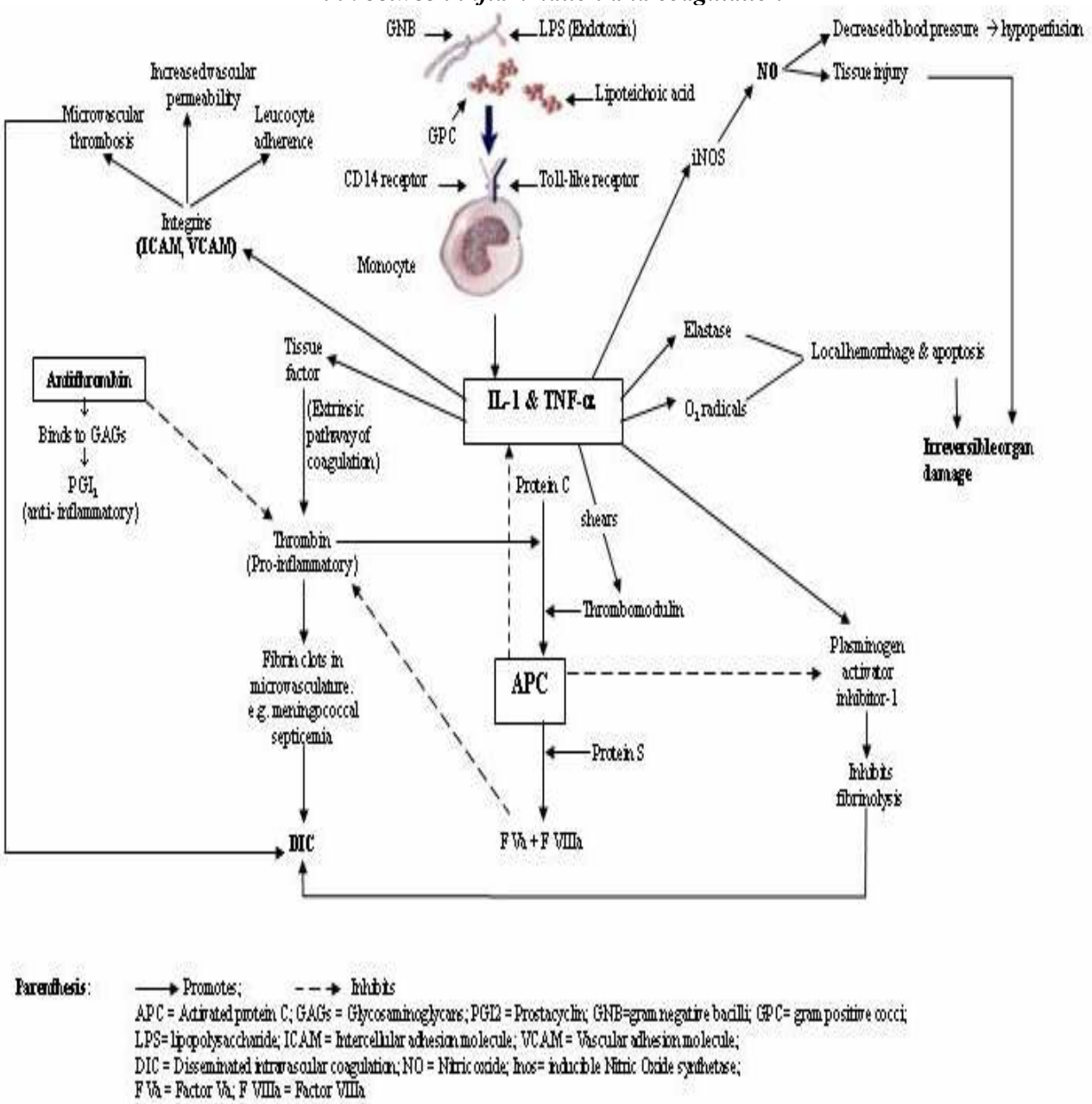


Figure 3

Algorithm - 1

Link between inflammation and coagulation is illustrated in the following algorithm-1:

Link between inflammation and coagulation



Parenthesis: → Promotes; --→ Inhibits

APC = Activated protein C; GAGs = Glycosaminoglycans; PGI2 = Prostacyclin; GNB=gram negative bacilli; GPC= gram positive cocci; LPS= lipopolysaccharide; ICAM = Intercellular adhesion molecule; VCAM = Vascular adhesion molecule; DIC = Disseminated intravascular coagulation; NO = Nitric oxide; Inos= inducible Nitric Oxide synthetase; F Va = Factor Va; F VIIIa = Factor VIIIa

Modulators of coagulation

Proinflammatory cytokines also disrupt the body's naturally occurring modulators of coagulation and inflammation, which are activated protein C (APC) and antithrombin. APC also restores fibrinolytic potential by inhibiting plasminogen activator inhibitor-1 [31]. In vitro studies reveal that APC has direct anti-inflammatory properties by inhibiting the production of pro-inflammatory cytokines by LPS-stimulated monocytes. It also inhibits leukocyte adhesion and accumulation.[32,33] Therefore APC has three in one function – antithrombotic, pro-fibrinolytic and anti-inflammatory. This has been well documented in a multicentre randomized controlled trial with rhAPC - the PROWESS study [Recombinant Human Activated Protein C (rhAPC) Worldwide Evaluation in Severe Sepsis] , which showed, that the administration of rhAPC reduced mortality from severe sepsis or septic shock by 6% compared with placebo.[34] Subsequently, rhAPC became the first drug approved by the FDA for the treatment of severe sepsis.[15,35] However, in another trial, the ADDRESS trial,[36] it was seen that in patients with a low risk of death, a 28 day mortality from all causes of sepsis was 18.5% on rhAPC as compared to17% on placebo. Hence it was suggested, that adult patients with sepsis induced organ dysfunction associated only with a clinical assessment of high risk of death, should receive rhAPC if not otherwise contraindicated. But adult patients with severe sepsis and low risk of death should not receive rhAPC. The ENHANCE trial[37] (open-label observational study) further suggested that early administration of rhAPC was associated with a better outcome. However serious bleeding occurred more often in patients treated with APC. Therefore, the decision on utilization depends upon assessing the likelihood of mortality reduction versus increases in bleeding and cost effectiveness.

Antithrombin is the second naturally occurring endothelial regulator affected during sepsis. Antithrombin inhibits thrombin production at multiple sites in the coagulation cascade as well as by directly binding and inhibiting thrombin.[38] Antithrombin, when bound to endothelial cell surface glycosaminoglycans (GAGs), leads to the production of the anti-inflammatory molecule prostacycline (PGI_2). Evidence exists that neutrophil elastase cleaves GAGs off the surface of the endothelial lining, thus limiting anti-inflammatory properties of antithrombin.[15]

SUMMARY

So, what happens in sepsis is a complex sequence of events that are responsible for the transition from sepsis to severe sepsis and septic shock.

- Circulating proteins interact with microbial products initiating a cascade of events leading to the production of inflammatory mediators – both proinflammatory as well as anti-inflammatory mediators.
- In addition, the complement system is activated, e.g. C5a and other products may promote neutrophil reactions like – chemotaxis, aggregation, degranulation and production of oxygen free radical. These reactions cause endothelial damage, neutropenia and hypotension.
- Coagulation proteins are activated by tissue factors expression on macrophage and endothelial cell.
- Fibrinolysis is inactivated by the loss of APC and thrombomodulin on endothelial cell surface.
- iNOS is transcriptionally activated leading to relaxation of tone of arteriolar smooth muscle.
- In the most exaggerated scenario, vasoregulatory dysfunction and micro aggregation impair microvascular flow, creating local ischemia and hypoxia, which may impair respiration [39,40]
- Anti-inflammatory mediators like IL-4, IL-10 and soluble inhibitors like soluble tumour necrosis factor receptor (sTNFR), IL-1 receptor antagonist (IL-1RA) are then produced by the body to mitigate these responses.

Hence in sepsis, especially early, the dominant proinflammatory response promotes coagulation over fibrinolysis leading to disseminated intravascular coagulation (DIC), ischemia, hypo perfusion and tissue injury. As time goes on, apoptosis of immune cells makes the patient more susceptible to opportunistic infections.[14] Structural increase in the permeability of the endothelium permit inflammatory cells and products to leave the circulation, leading to generalized edema.[16] Since endothelium is ubiquitous throughout the body, this " pan-

endothelial cell disruption" is central to the development of multisystem organ failure. In recent years, components of this host response have been the target of outcome trials for many therapies.

REFERENCES

1. Thomas L. Notes of a Biology Watcher: Germs. N Engl J Med, 287:553-555, (1972).
2. Bone RC, Balk RA, Cerra FB, et al. American College of Chest Physicians/Society for Critical Care Medicine Consensus Conference: Definitions for Sepsis and Organ Failure and Guidelines for the Use of Innovative Therapies in Sepsis. Chest, 101:1644-1655, (1992).
3. Munford RS. Sepsis and Septic Shock. In: Braunwald, Wilson, Fauci, Kasper, Jameson, Longo and Hauser (eds), *Harrison's Principles of Internal Medicine*. MaGraw-Hill, New York, 2001, pp 1695-1702.
4. Martin GS, Mannino DM, Eaton S and Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med, 348: 1546-1554, (2003).
5. Rangel-Frausto MS, Pitter D, Hwang T, Woolson RF and Wenzel RP. The dynamics of disease progression in sepsis: Markov modeling describing the natural history and the likely impact of effective antisepsis agents. Clin Infect Dis , 27: 185-190, (1998).
6. Annane D, Aegerter P, Jars-Guincestre MC, Guidet B. Current epidemiology of septic shock: the CUB-Rea Network. Am J Respir Crit Care Med, 168: 165-172, (2003).
7. Chamberlain NR septic Shock Gene Identified. 22nd January. Available at http://www.suite101.com/article.cfm/micribiology/14842. Accessed on 15th November 2008.
8. Sands KE, Bates DW, Lanken PN et al. Epidemiology of sepsis syndrome in 8 academic medical centers. Academic Medical Consortium Sepsis Project Working Group. JAMA, 278: 234-240, (1997).
9. Agnus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J and Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome and associated cost of care. Crit Care Med, 29(7): 1303-1310, (2001).
10. Alberti C, Burn-Buisson C, Buchardi H, et al. Epidemiology of sepsis and infection in ICU patients from an international multicentric cohort study. Intensive Care Med, 28: 108-121, (2002).
11. Sorensen TI, Neilsen GG, Andersen PK and Teasdale TW .Genetic and environmental influences on premature death in adult adoptees. N Engl J Med, 318(12):727-732, (1988).
12. Lin MT and Albertson TE. Genomic polymorphisms in sepsis. Crit Care Med, 32: 569-579, (2004).
13. Marzinger P. Tolerance, danger and the extended family. Annu Rev Immunol, 12: 991-1045, (1994).
14. Annane D, Bellissant E and Cavaillon JM. Septic Shock. Lancet, 365: 63-78, (2005).
15. LaRosa SP. Sepsis (Clevelandclinic Website) May 29th 2002. Available at http://www.clevelandclinicmeded.com/diseasemanagement/infectiousdisease/sepsis Accessed on 18th November 2008.
16. Matuschak GM. Circulating cytokine concentrations and outcome prediction in intensive care unit patients: still the tip of the iceberg? Crit Care Med, 24:1769-1771, (1996).
17. J.L.Vincent. Recent Developments in septic shock. In: A. P. Adams and J. N. Cashman (eds), *Recent Advances in Anaesthesia and Analgesia*. Churchill Livingstone, Singapore.
18. Young LS, Gascon R, Alam S and Bermuder LM. Monoclonal antibodies for treatment of gram negative infections. Rev Infect Dis, 11: S1564-1571, (1989).
19. Teng NN, Kaplan HS, Herbert JM et al. Protection against Gram negative bacteremia and endotoxemia with human monoclonal IgM antibodies. Proc Natl Acad Sci USA, 82; 1790-1794, (1985).
20. Greenman RL, Schein RMH, Martin MA et al. A controlled trial of E5 murine monoclonal IgM antibody to endotoxin in the treatment of Gram negative sepsis. JAMA, 266: 1097-1102, (1991).
21. Ziegler EJ, Fisher CJ, Sprung CL et al. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. N Engl J Med, 324: 429-436, (1991).

22. McCloskey RV, Straube RC, Sanders C et al. Chess Trial Study Group. Treatment of septic shock with human monoclonal antibody HA-1A. A randomized, double-blind, placebo controlled trial. Ann Intern Med, 121: 1-5, (1994).
23. Kohn FR, Natanson C, Alling DW et al. A controlled trial of HA-1A in a canine model of gram-negative septic shock. JAMA, 269: 2221-2227, (1993).
24. Srutz P and Liehl E. Lipid A analogs aimed at preventing the detrimental effects of endotoxin. Infect Dis Clin North Am, 5: 847-873, (1991).
25. McMillan DD and Boyd GN. The role of antioxidants and diet in the prevention and treatment of oxygen-induced lung microvascular injury. Ann N Y Acad Sci, 384:535-543, (1982).
26. Estrada C, Gomez C and Martin C. Nitric oxide mediates tumout necrosis factor alpha cytotoxicity in endothelial cells. Biochem Biophys Res Commun, 186: 475-482, (1992).
27. Cobb JP, Natanson C, Hoffman WD et al. *N*-amino-L-arginine , an inhibitor of nitric oxide synthase, raises vascular resistance but raises mortality rates in awake canines challenged with endotoxin. J Exp Med, 176: 1175-1182, (1992).
28. Cobb JP, Natanson C, Quezado ZMN et al. Lack of protection by *N*-Methyl-l-Arginine (NMA), a nitric oxide synthase inhibitor, in endotoxin (LPS)-challanged canines. Crit Care Med, 21: S280, (1993).
29. Preiser JC, Zhang H, Wachel D et al. Is the endotoxin-induced hypotention related to nitric oxide formation? Eur Surg Res, 26: 10-18, (1994).
30. Petros A, Bennett D and Vallance P. Effect of nitric oxide synthase inhibitors on patients with septic shock. Lancet, 338: 1557-1558, (1991).
31. Sakata Y, Loskutff DJ, Gladson CL, Hekman CM and Griffin JH. Mechanism of protein C dependent clot lysis: role of plasminogen activator inhibitor. Blood, 68: 1218-1223, (1986).
32. Mizutani A, Okajima K, Uchiba M and Noguchi T. Activated protein C reduces ischemia /reperfusion-induced renal injury in rats by inhibiting leukocyte activation. Blood, 95: 3781-3787, (2000).
33. Murakami K, Okajima K, Uchiba m, et al. Activated protein C prevents LPS-induced pulmonary vascular injury by inhibiting cytokine production. Am J Physiol, 272 (2Pt 1): L197-L202, (1997).
34. Rivers EP, McIntyre L, Morro DC and Rivers KK. Early and innovative interventions for severe sepsis and septic shock: taking advantage of a window of opportunity. CMAJ, 173(9); 1054-1077, (2005).
35. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez- Rodriguez A, et al. Recombinant human protein C Worldwide Evaluation in Severe Sepsis(PROWESS) Study group. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med:699-709, (2001).
36. Abraham E, Laterre PF, Garg R, et al. Administration of Drotrecogin Alfa (Activated) in Early Stage Severe Sepsis (ADDRESS) Study Group. Drotrecogin Alfa (Activated) for adults with severe sepsis and a low risk of death. N Engl J Med, 353: 1332-1341, (2005).
37. Vincent JL, Bernard GR, Beale R, et al. Drotrecogin Alfa (Activated) treatment in severe sepsis from the global open label trial ENHANCE: Further evidence for survival and safety and implications for early treatment. Crit Care Med, 33: 2266-77, (2005).
38. Rosenberg RD. Biochemistry of heparin Antithrombin interactions, and the physiologic role of this natural anticoagulant mechanism. Am J Med, 87: 2S-9S, (1998).
39. Hotchkiss RS and Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med, 348:138—150, (2003).
40. Aird WC. The role of endothelium in severe sepsis and multiple organ dysfunction syndrome. Blood, 101: 3765-77, (2003).