2. REVIEW OF LITERATURE

2.1 Haematological markers

2.2 Biochemical markers
   2.2.1 Proposed biomarkers in sepsis
   2.2.2 C-reactive protein

2.3 Severity of sepsis

2.4 Effect of antioxidant drugs on biomarkers of sepsis
2. REVIEW OF LITERATURE

In designing the methodology as well as while carrying out our study, we consulted several other related studies and articles which have been published in the last twenty years. Here is a synopsis of those studies under the following headings:

1. Haematological markers in sepsis
2. Proposed biochemical markers and C-reactive protein in sepsis
3. Severity of sepsis
4. Effects of antioxidant drugs on biomarkers in sepsis.

2.1 Haematological markers:

Early identification of sepsis can be difficult in severe burns because of the systemic changes that routinely accompany these burns. Housinger et al., (1993), in their review article examined the value of a falling platelet count in predicting the development of sepsis. Thirty-two pediatric patients who sustained lethal burn injuries were compared with 32 patients with similar burns who survived. Daily platelet count was evaluated in conjunction with clinical course. Thirty-one of the 32 non-survivors developed a platelet count less than \(0.1 \times 10^{12}/\text{L}\). Only 10 of the survivors had a similar occurrence. Platelet count decline preceded other signs of sepsis in all cases. A platelet count below \(0.1 \times 10^{12}/\text{L}\) for more than 4 days was uniformly associated with death. All patients who died succumbed to multisystem organ failure, consistent with sepsis. Their results emphasized platelet count as an independent predictor of sepsis and death.

Coagulation abnormalities and thrombocytopenia are common in severe sepsis, but sepsis-related alterations in platelet function are ill-defined. With this background Yaguchi et al., (2004), designed a study to elucidate the effect of sepsis on platelet aggregation, adhesiveness, and growth factor release. Agonist-induced platelet aggregation was measured in platelet-rich plasma separated from blood samples collected from 47 critically ill patients with sepsis of recent onset. Septic patients had consistently decreased platelet aggregation compared with controls, regardless of the platelet count, thrombin generation, or overt disseminated intravascular coagulation (DIC) status. The severity of sepsis correlated to the platelet aggregation defect. They concluded that sepsis decreases circulating platelets' hemostatic function, maintains
adhesion molecule expression and secretion capability, and modulates growth factor production.

Torkaman et al., (2009), conducted a retrospective study to identify the most common organisms causing sepsis and their associations with thrombocytopenia. They enrolled 53 eligible neonates whose blood culture yielded positively for any organism. The patients with Enterobacter spp. sepsis had a higher incidence of thrombocytopenia. The mortality rate was 15.1 percent (8/53 cases), which was significantly higher among those with the Enterobacter spp. sepsis (five cases, p-value is 0.033).

Kibe et al., (2011), in their review article mentioned that sepsis is a leading cause of mortality in critically ill patients. Delay in diagnosis and initiation of antibiotics have been shown to increase mortality. Procalcitonin (PCT) has emerged as the most studied and promising sepsis biomarker. For diagnostic and prognostic purposes in critical care, PCT is an advance on C-reactive protein and other traditional markers of sepsis, but is not accurate enough for clinicians to dispense with clinical judgement. Many other biomarkers are currently being investigated. To be highly useful in clinical practice, it may be necessary to combine these with other novel biomarkers and/or traditional markers of sepsis.

The WBC counts are usually less in elderly patients as compared to young adults. Aminzadeh and Parsa, (2011), found that even in elderly patients the WBC count is dramatically raised during infection. They conducted a study on 130 patients and found a significant correlation between the source of infection and WBC counts in the elderly group (P < 0.05), i.e. WBC count ≥ 14,000 was more common in urinary tract, gastrointestinal and skin infections. They also found a significant correlation between WBC count and age of the patients (P < 0.05) i.e. WBC ≥ 12,000 was more common in patients aged 45-64 years, and WBC < 4000 was more frequent in young patients aged less than 30 years.

2.2 Biochemical markers of sepsis:

2.2.1 Proposed biomarkers of sepsis
Oxygen free radicals may be implicated in the pathogenesis of both ischemia-reperfusion damage and in circulatory shock. The attack on the cell membrane by free radicals leads to lipid peroxidation, which can be assessed by the plasma malondialdehyde (MDA) level. With this concept Ben Baouali et al., (1994),
evaluated the importance of lipid peroxidation in critically ill patients. They enrolled nineteen patients at an early stage of circulatory shock, 11 patients in the weaning period of ventilation, 9 gastro-enterological patients without cardio-circulatory distress or sepsis, and 9 healthy volunteers. The MDA level was higher in critically ill patients than in control subjects (61% in patients with shock and 40% in patients on mechanical ventilation). No correlation was found between the MDA level and the outcome: multiple organ failure or acute respiratory distress syndrome.

Llesuy et al., (1994), did an animal study to evaluate the oxidative stress in muscle and liver. With the understanding that sepsis is an infection associated with systemic manifestations, they produced infection in rats by cecal ligation and double perforation. The activities of muscle antioxidant enzymes were found maximally diminished after 12 h of sepsis: 46% decrease for Mn-superoxide dismutase, 83% decrease for catalase, and 55% decrease for glutathione peroxidase. In liver, only catalase activity showed a 52% decrease after 24 h of sepsis. They concluded that oxidative stress appears to occur in skeletal muscle early at the onset of the septic syndrome, with inhibition of active mitochondrial respiration and inactivation of antioxidant enzymes.

Goode et al., (1995), designed a prospective, observational study comprising of sixteen patients with septic shock, defined as: a) clinical evidence of acute infection; b) hypo- or hyperthermia (< 35.6 degrees C or > 38.3 degrees C); c) tachypnea (> 20 breaths/min or being mechanically ventilated); d) tachycardia (> 90 beats/min); e) shock (systolic pressure < 90 mm Hg) or receiving inotropes. Fourteen patients also had secondary organ dysfunction. They observed that the antioxidant vitamin concentrations were significantly lower in the patients than the reference range obtained from a comparable group of healthy controls. They concluded that there was decreased antioxidant status in the face of enhanced free radical activity, and suggested potential therapeutic strategies involving antioxidant repletion.

Warner et al., (1995), studied the activities of SOD and CAT in the plasma and RBCs of septic patients to see if there were any significant differences in activity between septic patients and healthy controls. The prognostic potential of the antioxidant enzymes SOD and CAT was evaluated in sepsis. Enzyme concentrations were determined in samples obtained from septic patients at time of diagnosis. Statistically significant increases in activities of total plasma SOD (P <0.003, n = 32),
Review of literature

erthrocyte (RBC) SOD (P <0.007, n = 16), plasma CAT (P <0.0001, n = 32), and RBC CAT (P <0.005, n = 16) were found in septic patients when compared with healthy adult controls (n = 7). Further, within the group of septic patients, statistically significant differences were found for total plasma SOD (P <0.05) and plasma CAT (P <0.009) (but not for RBC determinations) when survivors (n = 15) were compared with non-survivors (n = 17). The most striking finding was that plasma total SOD values of >10 kU/L were found in 7 of 21(30%) patients who did not survive their sepsis and that these values did not overlap with any surviving patients or controls. However, while high total plasma SOD activity appears to have some potential as a prognostic indicator, the lower values (0.0-8.8 kU/L) do not.

Cowley et al., (1996), conducted a prospective, cohort study comprising of fifteen patients, who were within 16 hrs of development of severe sepsis and secondary organ dysfunction. The mean initial plasma antioxidant potential was lower than their range for healthy volunteers (p < 0.05). Survivors had an initial plasma antioxidant potential that was greater than non-survivors (p < 0.01), and serial subset analysis demonstrated that survivors, despite having a low initial plasma antioxidant potential rapidly attained normal or supranormal values. While plasma antioxidant potential also increased in non-survivors over time, values in this subset never reached the normal range and remained below values in survivors at all time points studied (p < 0.05). On the basis of their data they were of the opinion that plasma antioxidant potential initially decreases in patients with sepsis who develop organ dysfunction, and it increases over time.

Galley et al., (1996 a), designed a prospective observational study comprising of Fourteen patients who met the established criteria for sepsis syndrome with multiple organ dysfunction syndrome, and ten non-infected critically ill patients. They observed that Xanthine oxidase activity was increased in septic patients compared with both healthy volunteers (p < 0.01) and non-infected patients (p < 0.05), and was highest in the six patients who survived (p < 0.05). Lipid peroxides were increased in both septic patients (p < 0.001) and non-septic controls (p < 0.001). Xanthine oxidase activity did not relate to the Acute Physiology and Chronic Health Evaluation (APACHE) II score or to the presence of organ dysfunction. The mean ascorbyl radical concentration was increased in patients compared with healthy subjects (p <
0.05). They concluded that the patients with sepsis have xanthine oxidase activation, high free-radical concentrations, and evidence of free radical damage.

To compare the total plasma antioxidant capacity and selected individual antioxidants in patients with varying degrees of severity of sepsis Pascual et al., (1998), conducted a prospective, observational, consecutive case study. The study comprised of forty-six healthy controls, ten ICU patients, nine patients with systemic inflammatory response syndrome (SIRS), 11 septic patients, and 14 septic shock patients. They observed plasma antioxidant capacity to be lower in septic patients but higher in septic shock patients, as compared with controls. Bilirubin was the greatest contributor to the increase with shock, followed by uric acid. They concluded that although total plasma antioxidant capacity is decreased from normal levels in septic patients, an increase in some oxidants contributes to an increased total antioxidant capacity in septic shock patients.

Gutteridge and Mitchell, (1999), in their review article discussed the redox imbalance in critically ill patients. Experimental and clinical evidence demonstrates that these patients suffer from severe oxidative stress. In their research they have shown that the vascular pathology of sepsis/SIRS and ARDS is initiated through the uncontrolled production of reactive oxygen (ROS) and reactive nitrogen species (RNS) which modulate inflammatory cell adhesion and cause direct injury to endothelium.

Metnitz et al., (1999), designed an observational study to investigate the pattern of antioxidants in plasma and ROS production by neutrophils in patients with ARDS. Plasma levels of ascorbate, alpha-tocopherol, retinol, beta-carotene, selenium and lipid peroxidation products (MDA) were determined and the activities of the antioxidative enzymes CAT, SOD and GPX in erythrocytes were measured. In addition, ROS production (superoxide anion and hydrogen peroxide) in activated neutrophils was assessed. Plasma levels of alpha-tocopherol, ascorbate, beta-carotene and selenium were reduced from the onset of illness. MDA plasma levels were increased throughout the illness. ROS generation from neutrophils was normal on day 0, and decreased to day 6 in ARDS patients.

Batra and his colleagues, (2000), carried out a study to evaluate the status of antioxidant enzymes and non-enzymatic antioxidants with a view to suggesting the introduction of antioxidant therapy in neonatal sepsis. The activities of serum XO,
creatinine phosphokinase, SOD and GPX, and the content of MDA were found to be significantly elevated in the neonates with sepsis when compared with controls. Conversely, the activity of XO and the levels of uric acid and albumin were decreased. The septic, full-term neonates registered significantly higher CPK activity (70%) than the preterm septic neonates. However, infants with late-onset and septic shock had a significant decrease in CPK activity ($p < 0.05$) compared with their corresponding sub-groups. Likewise, UA levels were found to be 28% depressed ($p < 0.05$) in the babies with late-onset sepsis and 51% increased ($p < 0.001$) in babies with shock compared with their respective sub-groups. Neonates with septic shock also registered a significant elevation in GPx activity (28%) compared with those without shock.

Koo et al., (2000), conducted a study on animal model and found $\alpha$ GST (mean ± SEM, $30.5 \pm 3.5 \, \mu g/L$) level rise in the sham group at all measured time points. Although plasma levels of $\alpha$ GST did not change at 2 hours after caecal ligation and puncture (CLP), they were elevated by 24.9% at 5 hours after the onset of sepsis and continued to increase throughout the septic course. Plasma lactate levels were significantly increased only at 20 hours after CLP ($P < 0.01$). Previous studies have shown that liver transaminase levels did not increase at 5 hours, but at 10 and 20 hours after CLP. They concluded that since plasma $\alpha$ GST levels increased earlier than plasma lactate and liver transaminase levels, $\alpha$ GST may be a more sensitive indicator of early liver injury.

Koltuksuz et al., (2000), conducted a study to determine oxidative activities in the plasma of patients with Acute Appendicitis (AA). They measured SOD activities and MDA levels in samples from 31 patients diagnosed as having AA and 10 otherwise healthy children with inguinal pathologies. Both SOD and MDA were significantly higher in the Acute Supurative Appenditis (ASA) and Acute Perforated Appenditis (APA) groups compared to controls and Acute Focal Appenditis (AFA) group. The mean leukocyte numbers of the ASA and APA groups were significantly higher compared to the AFA group. Based to these results, they speculated that oxygen free radicals (OFR) may play an important role in the extent of AA.

Malmezat et al., (2000), studied the glutathione concentrations and synthesis rates in infected rats (2 d after infection) and in pair-fed controls. Glutathione synthesis rates were significantly greater in liver (+465%), spleen (+388%), large intestine (+109%),
l lung (+100%), muscle (+91%) and heart (+80%) of infected rats compared with pair-fed controls. Glutathione concentrations were also greater in these tissues but were unaffected in small intestine and lower in blood. In keeping with the stimulation of liver glutathione synthesis, the activities of liver γ-glutamyl-cysteine synthetase and glutathione reductase were significantly greater in liver of infected rats than of pair-fed rats.

Spolarics et al., (2001), observed the effect of type A-glucose-6-phosphate dehydrogenase (G6PDH) deficiency in trauma patients. In patients with severe injury, 50% of the deficient and 6.2% of non-deficient patients developed sepsis with positive bacterial blood cultures. In deficient patients, the frequency of bronchial (75%) and wound infections (25%) was also increased compared with non-deficient patients (32% and 0%). The durations of systemic inflammatory response syndrome, sepsis syndrome, and days on antibiotics were three times longer in deficient than in non-deficient individuals. However, adult respiratory distress syndrome occurred in 37% of both groups. After moderate injuries (Injury Severity Score, 9-16), the deficiency was not associated with adverse clinical effects, and the trauma-induced changes in leukocyte function were similar in deficient and non-deficient patients. They concluded that common type A-G6PDH deficiency predisposes septic complications and anemia in trauma patients after severe injuries.

Goodyear-Bruch and Pierce., (2002), in their review article highlighted that in critically ill patients, oxidative stress can induce inflammatory responses and cellular destruction and even lead to an increased mortality rate. They have emphasized on studies that have indicated a relationship between oxidative stress and common ICU syndromes and diseases affecting critically ill patients. Scavengers play an important role in terminating the activity of ROS. Use of antioxidants is also beneficial in preventing disease.

Alonso de Vega et al., (2002), did a prospective study to evaluate whether critically ill patients with systemic inflammatory response syndrome, on admission to an intensive care unit, had more severe oxidative stress than those without this syndrome. The study comprised of 68 adult patients admitted to the intensive care unit whose venous blood samples were routinely obtained within 24 hrs of admission to measure the plasma total antioxidant capacity, the lipid peroxidation products malondialdehyde and 4-hydroxynonenal, reduced sulfhydryl groups, and nitrites/nitrates. The patients
with criteria of SIRS (n = 20) had higher APACHE III scores (determined by collecting the worst value within 24 hrs after admission to the intensive care unit) and plasma concentrations of lipid peroxidation products and nitrites/nitrates and lower plasma concentration of reduced sulfhydryl groups and plasma total antioxidant capacity than patients without the syndrome (n = 48). Moreover, the markers for leukocyte activation, myeloperoxidase and polymorphonuclear elastase, presented higher concentrations in the plasma of patients with systemic inflammatory response syndrome.

Ritter et al., (2003), conducted a prospective study on 100 rats. The animals were randomly divided into three groups: sham-operated (n=20), cecal ligation and perforation resuscitated with normal saline (n=40), and cecal ligation and perforation with normal saline plus antibiotics (n=40). Blood samples were collected from all animals 3, 12, and 24 h after CLP through a jugular catheter inserted before CLP. Lipid peroxidation, protein carbonyls, and superoxide dismutase were significantly increased in nonsurvivor septic rats and were predictive of mortality. There was a marked increase in superoxide dismutase activity without a proportional increase in catalase activity in non-survivors. They concluded that plasma superoxide dismutase was an earlier marker of mortality.

The oxidant status of an individual is assessed by determining a group of markers in noninvasive samples. One limitation when measuring these biomarkers is that they do not give information about tissue localization of oxidative stress. Argüelles et al., (2004), undertook a study to establish whether the serum oxidative stress biomarkers are indicative of oxidative stress in tissues of an individual. To accomplish this, they determined a few generic markers of oxidation in serum and tissues of six groups of rats treated experimentally, to modulate their oxidative stress status. The correlation between serum and tissue levels was calculated for each marker. Also, for each tissue, the correlation between the values of these oxidative stress biomarkers was analysed. Their results show that only lipid peroxides in serum could be useful to predict the oxidative stress in tissues.

Rothet al., (2004), reviewed the antioxidative status in critically ill patients. Oxidative stress is caused by a higher production of reactive oxygen and reactive nitrogen species or a decrease in endogenous protective antioxidative capacity. In all types of critical illness, such as sepsis, trauma, burn injury, acute pancreatitis, liver
injury, severe diabetes, acute respiratory distress syndrome, AIDS and kidney failure, the occurrence of increased oxidative stress or a reduced antioxidative status was described. They summarised with the comment that occurrence of oxidative stress in critically ill patients is associated with a poor prognosis.

Zein et al., (2004), hypothesised that increased LDH in patients with severe sepsis and septic shock would reflect the extent of tissue injury and be associated with a worse prognosis. They conducted a retrospective analysis and collected clinical data and outcome variables from the medical records of 82 adult patients admitted to the intensive care unit with severe sepsis. The LDH level was elevated in 65 patients (79%) on admission. Increased LDH was associated with more physiologic abnormalities (higher APACHE II score), and organ failure (higher MODS score), and a higher lactic acid, SGOT, creatine kinase, creatinine, and lower platelet count and plasma bicarbonate level (p<0.05). There was significant correlation between LDH and lactic acid levels (r =0.76; p<0.01), and between LDH and MODS score (r =0.6; p<0.01). Death was associated with a higher LDH level (656±79; p<0.001 vs. 369±72 U/L). LDH levels that are increasing 48 hours after ICU admission (observed in 37 of 82 patients) were sensitive (0.72) and specific (0.77) in predicting mortality.

Kapoor et al., (2006), conducted a study to estimate the lipid peroxidation and antioxidant status in neonatal septicemia. It was a prospective study which included 44 septicemic babies as cases and a group of 84 matched healthy babies formed the control. MDA, SOD, GPX, catalase, uric acid (UA) and albumin (Alb) were estimated in the serum and compared between the groups. Septicemic panenotes had significantly higher levels of MDA, SOD, GPX, and catalase, while the levels of UA and Alb were significantly lower as compared to controls (p < 0.001). Significantly elevated levels of MDA (p < 0.05) and depressed levels of UA (p < 0.001) were found in babies with late onset sepsis. Neonates who ultimately succumbed had significantly elevated levels of MDA, SOD, GPX and catalase, whereas levels of UA and Alb were significantly depressed (p < 0.001).

Ozdogan et al., (2006), investigated the levels of total anti-oxidant status (TAS), as a marker of anti-oxidant defense system and MDA, as a marker of oxidative stress, in the plasma of patients with acute appendicitis. Blood samples for C-reactive protein (CRP), MDA and TAS were collected preoperatively. No significant differences in WBC counts and MDA levels between groups were encountered. Plasma CRP was
significantly higher in patients with perforated appendicitis, but not in the other groups. In advanced appendicitis group, TAS level was significantly lower than the other groups. On the other hand, plasma TAS level in acute phlegmonous appendicitis group was significantly higher. They concluded that a decrease in plasma total antioxidant capacity could be a predictor of the progression of inflammation to the perforation in acute appendicitis.

Supinski and Callahan, (2006), designed a study to test the hypothesis that superoxide scavenger administration prevents endotoxin-induced cardiac mitochondrial and contractile dysfunction. Endotoxin elicited increases in cardiac mitochondrial ROS formation (p<0.001), increases in cardiac levels of free radical reaction products, reductions in mitochondrial ATP generation (p<0.001), and decrements in cardiac pressure generating capacity (p<0.01). Administration of PEG-SOD blocked formation of free radical reaction products, prevented mitochondrial dysfunction and preserved cardiac contractility. They concluded with a remark that superoxide derived oxidants play a critical role in the development of cardiac mitochondrial and contractile dysfunction in endotoxin-induced sepsis.

Yerer et al., (2006), investigated the effects of melatonin on antioxidant enzyme activities in septic rats. They observed that GPX activity in untreated animals was 45.26 ± 6.81 (n = 10) nmol/gm Hb, while in LPS treated animals it was significantly increased to 76.41 ±7.67 (n = 10) nmol/gm Hb, indicating that the GPX enzyme activity increases in severe sepsis. The induction in the glutathione peroxidise activity was significant in septic rats treated with melatonin (92.94 ±10.41 nmol/gm Hb), compared to the control and melatonin groups. SOD activity was slightly increased when treated with melatonin alone, but this increase was not statistically significant. They therefore concluded that melatonin stimulates the antioxidant enzyme activities in the erythrocytes of septic rats.

Cherian et al., (2007), conducted a study on 38 children with sepsis (< 5 yr) and 39 age and sex matched control, to assess the levels of certain antioxidants in blood of children with sepsis. Red cell GSH, SOD and TBARS and plasma vitamin C were estimated by standard techniques. There was no significant change in erythrocyte GSH, SOD and TBARS levels in sepsis when compared to controls. This may be due to the adaptive response of the body to combat the oxidative stress. However, plasma vitamin C levels were significantly reduced in patients aged one year one month to
Review of literature

five years which may be due to active phagocytosis and due to its role as a free radical scavenger. They inferred that children affected by sepsis probably adapt to the free radical toxicity induced.

Wilmanski et al., (2007), evaluated the effect of G6PDH deficiency on the survival after endotoxemia or polymicrobial sepsis (cecal ligation and puncture). Lipopolysaccharide in vivo (lipopolysaccharide from Escherichia coli, 10-35 mg/kg body weight intraperitoneally) resulted in greater interleukin-1beta, interleukin-6, and interleukin-10 levels in serum and peritoneal lavage in G6PDH-deficient mice compared with wild type. It increased mortality in G6PDH-deficient animals (40-70%) as compared with wild type (5-40%). In contrast, mortality after cecal ligation and puncture-induced sepsis was similar in G6PDH-deficient and wild-type animals either in saline-resuscitated or antibiotic-treated animals. They concluded that G6PDH deficiency augments cytokine responses after inflammatory challenges. The deficiency is disadvantageous as reflected in increased mortality after hyperinflammation caused by acute endotoxemia. However, the deficiency may not manifest worsened survival after the immunosuppressed condition associated with severe sepsis.

Büyükbaş et al., (2008), evaluated the status of oxidative stress and antioxidant in 25 mixed critically ill patients with respiratory failure as assessed by the MDA, NO, SOD, GPX, vit C, vit E and uric acid measurements in bronchoalveolar lavage (BAL), erythrocyte and plasma. Twenty five patients without RF was evaluated as control group. The mean MDA levels of plasma, erythrocyte and BAL fluid in mixed critically ill with respiratory failure were higher than control group. The mean NO levels of plasma and BAL fluid in mixed critically ill with respiratory failure were higher than control group. The mean SOD levels of plasma, erythrocyte and BAL fluid in mixed critically ill with respiratory failure were lower than control group. The mean of uric acid, vit C, vit E levels of plasma in mixed critically ill with respiratory failure were lower than control group. The mean of GPX level of erythrocyte in mixed critically ill with respiratory failure was lower than control group.

Andrades et al., (2011), conducted a study to determine in situ oxidative and inflammatory markers and correlate those with markers of organ failure. The study comprised of rats subjected to CLP, treated with basic support or antioxidants and killed 12 h after to determine thiobarbituric acid reactive species (as an index of
oxidative damage), SOD, catalase, and myeloperoxidase (MPO) (as an index of neutrophil infiltration) in the kidney and lung. The authors found a positive correlation between TBARS and markers of organ injury in lung and kidney. Oxidative damage was correlated with an increase in SOD/CAT ratio only in the lung. They concluded that despite the general occurrence of oxidative damage in different organs during sepsis development and a positive correlation between oxidative markers and organ injury.

2.2.2 C-reactive protein in sepsis

Matson et al., (1997), assessed the changes in the plasma concentration of C-reactive protein as a diagnostic test for sepsis in critically ill patients. Forty-nine episodes of secondary sepsis were identified in 31 patients. In 43 out of the 49 episodes there was a 25% or greater change in the concentration of C-reactive protein on the day that sepsis was diagnosed but in six episodes of sepsis the change was less than 25%. They concluded that a 25% rise in the plasma concentration of C-reactive protein in the absence of other non-infective causes of a raised C-reactive protein, such as inflammation, tissue injury or surgery, is highly suggestive of infection, but failure of the C-reactive protein to rise does not eliminate a diagnosis of sepsis.

To determine the use of plasma C-reactive protein (CRP) concentrations, body temperature (BT) and white blood cell count (WBC) in the detection of sepsis in critically ill patients. Póvoa et al., (1998) designed a prospective study in all patients admitted for more than 24 h in the intensive care unit (ICU). A total of 306 patient-days were analysed for the infection status, of which 23 patients were followed: 20 Negative, 15 Definite, 63 Unlikely and 208 Probable. The median (range) CRP values for Negative, Unlikely, Probable and Definite groups were as follows: 24.5 (7-86), 34 (5-107), 143 (39-544), and 148 (52-320) mg/l. The plasma CRP levels were significantly related to the infectious status (Negative, Unlikely, Probable or Definite) of the patient-day classification (p < 0.05). Concentrations of CRP in the Negative and Unlikely groups were significantly lower than in the Probable and Definite ones (p < 0.05). A plasma CRP of 50 mg/l or more was highly suggestive of sepsis (sensitivity 98.5%, specificity 75%). They concluded that daily measurement of CRP was useful in the detection of sepsis and it was more sensitive than the markers, such as BT and WBC.
Meisner et al., (1999), analysed the relation of PCT plasma concentrations compared with CRP in patients with different severity of multiple organ dysfunction syndrome (MODS) and systemic inflammation. The study comprised of 40 patients with systemic inflammation and consecutive MODS, in whom PCT, CRP, the sepsis-related organ failure assessment (SOFA) score, the Acute Physiology, Age, Chronic Health Evaluation (APACHE) II score and survival were evaluated over a period of 15 days. They observed that higher SOFA score levels were associated with significantly higher PCT plasma concentrations (SOFA 7-12: PCT 2.62 ng/ml, SOFA 19-24: PCT 15.22 ng/ml) (median), whereas CRP was elevated irrespective of the scores observed (SOFT 7-12: CRP 131 mg/l, SOFT 19-24: CRP 135 mg/l). They concluded that measurement of PCT concentrations during multiple organ dysfunction syndrome provided more information about the severity and the course of the disease than that of CRP.

Brunkhorst et al., (2000), designed a prospective cohort study including all consecutive patients admitted to the ICU with the suspected diagnosis of infection. PCT values were highest in patients with septic shock (12.89+/−4.39 ng/ml; P<0.05 vs patients with severe sepsis). Patients with severe sepsis had significantly higher PCT levels than patients with sepsis or SIRS (6.91+/−3.87 ng/ml versus 0.53+/−2.9 ng/ml; P<0.001, and 0.41+/−3.04 ng/ml; P<0.001, respectively). APACHE-II scores did not differ significantly between sepsis, severe sepsis and SIRS (19.26+/−1.62, 16.09+/−2.06, and 17.42+/−1.72 points, respectively), but was significantly higher in patients with septic shock (29.27+/−1.35, P<0.001 versus patients with severe sepsis). Neither CRP, cell counts, nor the degree of fever showed significant differences between sepsis and severe sepsis, whereas white blood cell count and platelet count differed significantly between severe sepsis and septic shock. In contrast to APACHE-II, PCT appeared to be a useful early marker to discriminate between sepsis and severe sepsis.

Blommendahl et al., (2002), analysed serum CRP and the blood immature to total neutrophil leucocyte ratio in neonates. All had reasonable (58-77%) sensitivity, reasonable (62-84%) specificity, good (94-97%) negative predictive value and poor (16-24%) positive predictive value for the diagnosis of sepsis. They were of the opinion that instead of single test a combination of various tests would produce improvements in sensitivity or specificity. CRP is an acute-phase protein, the blood
levels of which increase rapidly in response to infection, trauma, ischemia, burns, and other inflammatory conditions. Although used frequently in the ICU as a sepsis marker, the relation of CRP levels to organ damage is not well known. 

Lobo et al., (2003), in a prospective study found that patients with high CRP levels at ICU admission had more severe organ dysfunction (higher sequential organ failure assessment scores, days of renal extracorporeal support therapy), longer ICU stays, and higher mortality rates than patients with normal ICU admission CRP levels. CRP concentrations were correlated with the presence and number of organ failures. ICU admission serum CRP levels > 10 mg/dL were associated with a significantly higher incidence of respiratory (65% vs 28.8%, p < 0.05), renal (16.6% vs 3.6%, p < 0.05), and coagulation (6.4% vs 0.9%, p < 0.05) failures, and with higher mortality rates (36% vs 21%, p < 0.05) than CRP levels < 1 mg/dL. In patients with CRP concentrations > 10 mg/dL on ICU admission, a decrease in CRP level after 48 h was associated with a mortality rate of 15.4%, while an increased CRP level was associated with a mortality rate of 60.9% (relative risk, 0.25; 95% confidence interval, 0.07 to 0.91; p < 0.05).

To compare the clinical informative value of PCT and CRP plasma concentrations in the detection of infection and sepsis and in the assessment of severity of sepsis Luzzani et al., (2003), classified 800 patients into the four categories. The median plasma PCT concentrations in non-infected (systemic inflammatory response syndrome) and localized-infection patient days were 0.4 and 1.4 ng/mL (p <.0001), respectively; the median CRP plasma concentrations were 79.9 and 85.3 mg/L (p =.08), respectively. The area under the receiver operating characteristic curve was 0.756 for PCT (95% confidence interval [CI], 0.675-0.836), compared with 0.580 for CRP (95% CI, 0.488-0.672) (p <.01). The median plasma PCT concentrations in nonseptic (systemic inflammatory response syndrome) and septic (sepsis, severe sepsis, or septic shock) patient days were 0.4 and 3.65 ng/mL (p <.0001), respectively, whereas those for CRP were 79.9 and 115.6 mg/L (p <.0001), respectively. The area under the receiver operating characteristic curve was 0.925 for PCT (95% CI, 0.899-0.952), compared with 0.677 for CRP (95% CI, 0.622-0.733) (p <.0001). The linear correlation between PCT plasma concentrations and the four categories was much stronger than in the case of CRP (Spearman's rho, 0.73 vs. 0.41; p <.05). A rise in sepsis-related organ failure assessment score was related to a higher
median value of PCT but not CRP. They therefore concluded that PCT was a better marker of sepsis than CRP. The course of PCT showed a closer correlation than that of CRP with the severity of infection and organ dysfunction.

Castelli et al., (2004), hypothesised that PCT and CRP concentrations are different in patients with infection or with no infection at a similar severity of organ dysfunction or of systemic inflammatory response. They assessed one hundred and fifty adult ICU patients consecutively over a period of 10 days. PCT, CRP and infection parameters were compared among the following groups: no systemic inflammatory response syndrome (SIRS) (n = 15), SIRS (n = 15), sepsis/SS (n = 71) (including sepsis, severe sepsis and septic shock [n = 34, n = 22 and n = 15]), and trauma patients (n = 49, no infection). They observed that PCT and CRP concentrations were higher in patients in whom infection was diagnosed at comparable levels of organ dysfunction, although correlation with the SOFA score was weak ($R = 0.254, P < 0.001$ for PCT, and $R = 0.292, P < 0.001$ for CRP). CRP levels were near their maximum already during lower SOFA scores, whereas maximum PCT concentrations were found at higher score levels (SOFA score > 12). PCT and CRP concentrations were 1.58 ng/ml and 150 mg/l in patients with sepsis, 0.38 ng/ml and 51 mg/l in the SIRS. They concluded that PCT and CRP levels were related to the severity of organ dysfunction, but concentrations were still higher during infection.

CRP has been considered a marker for infection and an aid for diagnosing sepsis. Seller-Perez et al., (2005), analyzed the relation of CRP to infection and outcome in intensive care units patients. They conducted a prospective study on 77 ventilated patients with confirmed infection at admission. 55 admissions after elective surgery were the controls. CRP measurement the first (CRP-1), third (CRP-3) and sixth (CRP-6) day of stay. APACHE II, SOFA (Sepsis-related Organ Failure Assessment), shock, respiratory or renal failure, leucocytes, platelets and albumin were registered. Mortality was 23.4% in cases and 1.8% in controls. Age, shock, APACHE II and SOFA were related to mortality, but CRP-1 did not. They concluded that CRP level on admission is an useful marker for early infection but not for outcome in critically ill patients admitted to the ICU.

To assess the value of CRP after prescription of antibiotics in order to define clinical resolution of community-acquired sepsis (CAS) admitted to the ICU Povoa et al., (2011) conducted a cohort multiple-centre observational study in 17 Portuguese ICUs.
Patients were followed-up during the first 5 ICU days, the day of ICU discharge or death and hospital outcome. Comparison between survivors and non-survivors was performed. When they compared CRP of survivors and non-survivors at the different time points, they found that CRP of non-survivors was significantly higher since D3 onwards ($P < 0.001$, for day 3, day 4 and day 5). A patient with an average decrease of the CRP concentration of 10% per day has 32% less chance of dying when compared with a patient with the same SAPS II and the same severity of sepsis but with no decrease of the CRP. They concluded that daily CRP measurement after antibiotic prescription was useful in identification, as early as day 3, of CAS patients with poor outcome.

2.3 Severity of sepsis:

Many types of severity or prognostic scoring systems exist for intensive care unit (ICU). APACHE II is well established and widely used system for scoring severity of illness in ICU. The objectives of such scoring system are to undergo audit and to compare the different treatment modalities in those with similar severity of illness. Lee et al., (1993), conducted a study on 131 patients admitted in the ICU with an average length of stay of 4.1 days and mechanical ventilation of 62%. The average APACHE II score was 21.5 ± 12.1 (range 2-55). Their survey validated that APACHE II scoring system for severity of illness allows reliable prediction of outcome in ICU. Their results indicated that the aged fared as well as their younger counterparts. Patient with sepsis fared poorer than expected from their APACHE II score and stroke patients who needed ICU admission had an extremely poor prognosis (mortality of 85.7%).

To determine the relation between plasma redox status and severity of illness for patients admitted to an intensive care unit (ICU) Alonso de Vega et al., (2000), designed a prospective cohort study comprising of a total of 73 consecutive patients admitted to the ICU. The plasma ratio total antioxidant capacity (mM)/lipoperoxides was used as an index of plasma redox status. Analysis of correlation between plasma ratio total antioxidant capacity/lipoperoxides and APACHE III score showed a negative association ($p < .001$, Spearman correlation test). Myeloperoxidase and polymorphonuclear-elastase correlated positively with Acute Physiology and Chronic Health Evaluation III scores ($r^2 = 0.58$; $p < .001$; and $r^2 = 0.05$; $p = .035$; respectively). They concluded that plasma redox status relates to severity in critically
ill patients. Plasma redox status might become useful to evaluate the risk in critically ill patients.

Cui et al., (2004), hypothesized that severity of sepsis would also influence inhibition of superoxide anion, another inflammatory mediator. To test this, 6-h infusions of M40401, a selective SOD mimetic, or placebo were given to antibiotic-treated rats (n= 547) starting 3 h after challenge with differing doses of intravenous Escherichia coli designed to produce low- or high-control mortality rates. Their data indicated that preclinical sepsis models, possibly related to divergent effects on vascular function, inhibition of superoxide anion improved survival with more severe sepsis and high-control mortality rates but was less effective or harmful with less severe sepsis.

Siddiqui et al., (2004), did a retrospective review, at the correlation between APACHE II scores of patients admitted to their ICU within twenty four hours and the development of type of infection as well as evidence of hemodynamic involvement (i.e. presence of sepsis). They found that patients with increased APACHE II scores had moderate to severe signs and symptoms of sepsis including hemodynamic complications, increased respiratory rate, temperature changes and mental status changes.

Chuang et al., (2006), conducted a prospective observational study to investigate whether serum Total antioxidant capacity (TAC) levels were elevated or suppressed in emergency department patients with severe sepsis. The APACHE II score was used for clinical evaluation of the severity of sepsis. Serum TAC levels in patients with severe sepsis correlated positively with APACHE II scores \( r = 0.426, 95\% \text{ confidence interval [CI]} 0.2–0.6; P <0.001 \). After controlling for age and serum creatinine level, TAC still exhibited a positive correlation with APACHE II score \( P = 0.027 \). Serum TAC levels were higher in non-survivors than in survivors \( 812.0 \pm 322.4 \mu\text{mol/L versus 575.4 \pm 254.6 \mu\text{mol/L, 95\% CI 91.2–382.0; P = 0.002}} \). Serum TAC levels in patients with severe sepsis were significantly and positively correlated with serum UA levels \( r = 0.726, 95\% \text{ CI 0.595–0.819; P <0.001} \). Serum UA exhibited a weak but significant correlation with APACHE II score \( r = 0.306, 95\% \text{ CI 0.082–0.501; P = 0.009} \). However, there was no significant correlation of serum albumin or bilirubin level with APACHE II score. They concluded that elevated serum TAC level may reflect clinical severity of sepsis.
Kumar et al., (2007), conducted a prospective study to evaluate the production of superoxides and endogenous antioxidants in forty-five patients undergoing surgery for secondary peritonitis. Severity of illness at admission (APACHE II score) was correlated with admission levels of superoxide radicals and antioxidants (SOD, catalase and glutathione peroxidase). Superoxide and antioxidant levels at admission (day 1) and post-operative days 3 and 5/7 were then correlated with outcome. Nine of the 45 patients died (20% mortality) and 17 patients had complications (47% morbidity). The mean APACHE II score on admission was significantly higher among non-survivors than survivors (p < 0.01). The APACHE II score on admission correlated with the level of free radicals (r = 0.477, p < 0.01), catalase (r = -0.489, p < 0.01) and SOD (r = -0.357, p < 0.05).

Silvestre et al., (2009), designed a prospective study including all patients with sepsis admitted to an ICU. Patients were categorized into sepsis, severe sepsis and septic shock. APACHE II score, Simplified Acute Physiology Score (SAPS) II, Sequential Organ Failure Assessment (SOFA) score, CRP, body temperature and white cell count (WCC) of the day of sepsis diagnosis were collected. No correlation was found between CRP concentrations and severity of sepsis. They concluded that in septic patients, CRP on the day of sepsis diagnosis is not a good marker of prognosis.

López et al., (2011), conducted study on 39 patients with sepsis and correlated the levels of PCT and CRP with the severity scale of APACHE II. Correlation of mortality/APACHE II r = 0.707 p = 0.01; PCT/APACHE II r = 0.523 p = 0.001. For cultures: CRP/culture r = 0.575 p = 0.0001, PCT/culture r =0.448 p = 0.004. Relative risk (RR): PCT > than 2 ng/dl and cultures RR= 4. The relative risk PCT >2 mg/dl and death RR= 3.3. Cultures and CRP>128 RR= 2.4; death and CRP > 128 mg/dl RR= 2. They concluded that PCT and CRP values are useful markers to determine early gravity of an infectious illness.

Mühl et al., (2011), hypothesized that oxidative stress and leukocyte activation markers can lead to the severity of sepsis. The pro-oxidant parameters were significantly elevated in sepsis patients at admission, ROS intensity increased in burn patients until the 5th day. Endogenous antioxidant levels except catalase showed increased levels after burn trauma compared to sepsis. Elevated granulocyte activation and suppressed lymphocyte function were found at admission and early activation of granulocytes caused by increasing activation/migration markers in sepsis. They
concluded that severe sepsis was accompanied by oxidative stress and pathological leukocyte endothelial cell interactions.

Severe sepsis and septic shock are the leading causes of morbidity and mortality in the intensive care unit. With the hypothesis that biomarkers may help in risk stratification and prediction of mortality in these patients Taneja et al., (2011), conducted a prospective observational study to assess the trends of PCT, CRP and interleukin-6 (IL-6) values in patients with severe sepsis and septic shock and correlated those with 28-day mortality. The APACHE II score was significantly higher in non-survivor group with area under curve (AUC) 0.765 (95% CI 0.66, 0.86, p=0.000). Among the 3 biochemical markers at admission, AUC of IL-6 0.664 (95% CI 0.556, 0.772, p=0.005) was closest to that of APACHE II score. All parameters showed declining trends during the first 72 hours. They concluded that APACHE II score was a good predictor of mortality in patients with severe sepsis.

2.4 Effect of antioxidant drugs on biomarkers in sepsis:

Excessive inflammatory responses and impaired oxygen utilization because of microcirculatory failure are implicated in septic shock. Recent studies have pointed out some beneficial effects in the treatment of septic shock of several vasodilators that exert anti-inflammatory properties. In particular, the antioxidant N-acetylcysteine (NAC) has been demonstrated to enhance cardiac performance, and to improve hepatosplanchnic perfusion and liver function in patients with established septic shock. These clinical observations may lead us to examine further the role of antioxidant agents in developing novel therapies for septic shock.

NAC, a substrate for the production of glutathione, has potent antioxidant effects. As a nitrosothiol, it may also improve capillary blood flow. Bakker et al., (1994), studied the effects of NAC in a dog model of endotoxic shock. Blood lactate levels fell more rapidly in the NAC dogs than in the control dogs. Blood lactate levels returned to normal in the NAC dogs but not in the control dogs. Tumor necrosis factor (TNF) also decreased significantly in the NAC dogs but remained elevated in the control dogs. Their data indicated that NAC administration in endotoxic shock was well tolerated, could increase oxygen availability to the tissues, and was associated with an attenuation of TNF release.
Villa and Ghezzi, (1995), studied the effect of the antioxidant NAC in a model of polymicrobial sepsis induced in mice by cecal ligation and puncture. NAC significantly improved survival during the 6 days following sepsis induction and caused lower liver toxicity. Their results showed that the induction of xanthine oxidase was not deleterious in their model of sepsis and suggested that NAC works as a direct antioxidant and scavenger of free radicals generated from other sources.

Rojas et al., in 1996, studied the effect of acute endotoxin-induced septic shock on myocardium oxidative stress after low or high vitamin C and/or E dietary supplementation in guinea pigs. Vitamin C supplementation increased heart ascorbate whereas endotoxic shock totally depleted the heart ascorbate of vitamin C supplemented animals without changing vitamin E. Their results suggested that ascorbate was a primary antioxidant target in the heart of endotoxin treated mammals lacking the capacity to synthesize ascorbate and that ascorbate can have a protective value against endotoxin-induced free radical damage in the myocardium.

Bernard et al., (1997), conducted a randomized, double-blind, placebo-controlled, prospective clinical trial in patients meeting a predetermined definition of ARDS and requiring mechanical ventilation. The patients received standard care for ARDS and IV infusion, every 8 hourly for 10 days, either NAC (70 mg/kg, n=14), L-2-oxothiazolidine-4-carboxylate (OTZ) (63 mg/kg, n=17), or placebo (n=15). Both antioxidants effectively repleted RBC glutathione gradually over the 10-day treatment period. The NAC and OTZ treatment groups had substantial increased over baseline values of 47% and 49% compared to placebo, with maximum values of 2,366±171 nM/mL and 2,044±171 nM/mL (SEM), respectively. Maximum values were obtained on day 10 in both treatment groups. They concluded that NAC and OTZ may have the ability to safely replete glutathione in patients with ARDS and acute lung injury, with the potential to combat oxidant-induced damage.

Galley et al., (1997), investigated the effect of intravenous antioxidant therapy on antioxidant status, lipid peroxidation, hemodynamics and nitrite in patients with septic shock. Thirty patients randomly received either antioxidants (NAC 150 mg/kg for 30 min then 20 mg/kg/h plus bolus doses of 1 g ascorbic acid and 400 mg tocopherol) or 5% dextrose. Basal vitamin C was low and redox-reactive iron was elevated in all patients. In the 16 patients receiving antioxidants, vitamin C increased but total antioxidant capacity was unaffected. Lipid peroxides were elevated in all patients but
Review of literature

did not increase further in the patients receiving antioxidants. They concluded that antioxidant administration may be a useful adjunct to conventional approaches in the management of septic shock

Molnar et al., (1999), conducted a prospective, randomized, double blind trial on 100 patients who required mechanical ventilation and the support of two or more organ systems. The eligible patients were divided into two groups. One group received NAC and the other received an equal volume of 5% dextrose as placebo. The dose of NAC was given as a bolus of 150 mg/kg in 250 ml of 5% dextrose followed by an infusion of 12 mg/kg/h in 500 ml of 5% dextrose for 24 hours. Infusion was continued for three to five days. They concluded that NAC treatment made no significant difference in outcome in the ICU population. They mentioned that in animal models of sepsis, NAC pre-treatment has been shown to significantly suppress neutrophil oxidative burst activity and cause delayed bacterial elimination.

Ortolani et al., (2000), examined whether the antioxidants GSH and NAC play a protective role against damage by OFR in early septic shock. They randomly enrolled 30 patients with septic shock into one of three groups within 24 h of diagnosis. All of the patients received septic shock therapy, including parenteral nutrition, antibiotics, and volume-expanding and inotropic agents. One group (Group B) also received 70 mg/kg/d of intravenous GSH, and a second group (Group C), 70 mg/kg/d of intravenous GSH and 75 mg/kg/d of intravenous NAC. A significant decrease in peroxidative indexes was observed at day 5 in Group B as compared with both the control group and basal values. The decrease in peroxidative indexes was even more marked in Group C. Clinical scores in this group were also significantly improved. They concluded that administration of high doses of NAC added to GSH significantly decreased the peroxidative stress of patients with septic shock.

Victor et al., (2000), studied the in vitro effect of ascorbic acid, at different concentrations (0.001, 0.01, 0.1, 1 and 2.5 mM), on the various steps of the phagocytic process, i.e., adherence to substrate, chemotaxis, ingestion of particles and superoxide anion production of murine peritoneal macrophages obtained from mice with that of endotoxic shock, at 2, 4, 12 and 24 h after LPS injection. Their result suggested that ascorbic acid can regulate the phagocytic process in endotoxic shock, principally decreasing free radical production and thus it could reduce endotoxic shock severity.
Nathens et al., (2002), conducted a randomized, prospective study to compare outcomes in patients receiving antioxidant supplementation (alpha-tocopherol and ascorbate) versus those receiving standard care. The relative risk of pulmonary morbidity was 0.81 (95% confidence interval 0.60-1.1) in patients receiving antioxidant supplementation. Multiple organ failure was significantly less likely to occur in patients receiving antioxidants than in patients receiving standard care, with a relative risk of 0.43 (95% confidence interval 0.19-0.96). They concluded that early administration of antioxidant supplementation using alpha-tocopherol and ascorbic acid reduces the incidence of organ failure and shortens ICU length of stay.

Soybir et al., (2002), conducted a study to determine the effects of vitamin E and the iron chelating agent desferrioxamin (Dfx), supplemented by clindamycin and gentamycin therapy, on peritonitis caused by caecal ligation of a puncture wound in an experimental model. In the control groups, mortality at 14 days was 66%. Rats treated with antibiotics alone (Group 5) had a mortality rate of 40%. Those treated with a combination of antibiotics and vitamin E (Group 7), however, had a mortality rate of only 14%, and those treated with antibiotics and Dfx had a mortality rate of only 7%. Their study suggested that treatment of peritonitis in rats with a combination of Dfx and antibiotics had a significant beneficial effect on survival, in comparison with treatment with antibiotics alone.

Molnar et al., (2003), conducted a prospective, randomised, double-blinded, placebo-controlled clinical trial on 100 patients. The treatment group (n=47) received NAC (150 mg/kg bolus followed by a continuous infusion of 12 mg/kg/h and the placebo group (n=46) received the same volume of 5% dextrose during surgery. Serum PCT, CRP and microalbuminuria was monitored preoperatively, on admission to ICU, then daily during the first 3 postoperative days. Significantly lower CRP levels were found in the NAC group on days 1 and 2 (NAC vs. placebo respectively). They concluded that short-term NAC treatment decreased CRP levels, but failed to attenuate any other inflammatory response, as monitored by serum PCT and microalbuminuria. Overall, their results did not support the routine prophylactic use of NAC as a free radical scavenger in abdominal surgery.

Patterson et al., (2003), conducted a prospective, randomized, double blind, placebo-controlled pilot trial on twenty patients with sepsis. Nuclear factor-kappa B activation was measured in mononuclear leukocytes at baseline and 24, 48, 72, and 96 hrs later.
Activation decreased significantly in patients treated with NAC (p = .016) but not placebo and was significantly reduced at 72 hrs compared with both preinfusion values (p = .028) and patients receiving placebo (p = .01). They were of the opinion that antioxidant therapy with NAC may be useful in blunting the inflammatory response to sepsis.

Victor, et al., (2003), conducted a study to evaluate the effect of the administration of the antioxidant N-acetylcysteine (NAC) on the redox state of peritoneal macrophages and lymphocytes from mice with lethal endotoxic shock (100 mg/kg i.p. of lipopolysaccharide, LPS). The injection of NAC (150 mg/kg i.p. at 30 min after LPS injection) decreased the ROS, the TNF alpha, the MDA levels, iNOS expression and the GSSG/GSH ratio, and increased the antioxidant defenses in both macrophages and lymphocytes. Moreover, the NAC treatment prevented the activation of nuclear translocation of the nuclear factor kappa B, which regulates ROS, inflammatory cytokines and antioxidant levels. They concluded that antioxidants could offer an alternative treatment of human endotoxic shock.

Bhattacharyya et al., (2004), in their review article on the mode of action of endotoxin, evaluated the role of oxygen radicals or reactive oxygen species in the pathogenetic sequence of reactions mediated by endotoxin (LPS) leading to the production of sepsis. As per their review, among reactive oxygen species hydroxyl radical either singly or in combination with peroxynitrite, produces tissue damage often observed during septic injury. Inactivation of these damaging radicals by antioxidants or nitric oxide inhibitor(s) may be helpful for protecting sepsis mediated derangements.

Emet et al., (2004), evaluated the effects of continuously infused NAC on serum cytokine levels and gastric intramucosal pH in humans suffering from severe sepsis. They found that NAC infusion at the doses given did not affect cytokine levels, outcomes, or gastric intramucosal pH in patients with severe sepsis. However, they were of the opinion that because of the limited number of patients included in the study and the short period of observation, their findings needed confirmation in larger clinical trials of NAC infused in a dose-titrated manner.

Ritter et al., (2004), conducted a prospective, randomized, controlled experiment on rats subjected to CLP. The subjects were treated with either N-acetylcysteine (20
mg/kg, 3 hrs, 6 hrs, 12 hrs, 18 hrs, and 24 hrs after CLP, subcutaneously) plus deferoxamine (20 mg/kg, 3 hrs and 24 hrs after CLP, subcutaneously) or vehicle with or without “basic support” (saline at 50 mL/kg immediately and 12 hrs after CLP plus ceftriaxone at 30 mg/kg and clindamycin 25 mg/kg every 6 hrs). Mitochondrial superoxide production was significantly reduced by antioxidant treatment. Furthermore, antioxidants significantly improved the balance between catalase and superoxide dismutase activities. Survival in untreated septic rats was 10%. Survival increased to 40% with fluids and antibiotics. In rats treated only with NAC plus deferoxamine, survival was also significantly improved (47%) in a manner similar to basic support. Survival increased to 66% with basic support with NAC plus deferoxamine.

Spapen, (2004), discussed the possible role of NAC in sepsis. In the review he mentioned that of the new advent NAC shows great promise. Beside proven antioxidant, anti-inflammatory and cytoprotective effects, NAC does also ensure endothelial protection and enhances microvascular blood flow. Studies that put these highly favourable properties to the clinical test remain scarce but are definitely needed to determine whether NAC has a place in our therapeutic armamentarium against sepsis.

Akinci et al., (2005), conducted a prospective randomized, double-blind, placebo controlled study. Twenty-six patients were randomized to receive either NAC in 5% dextrose 40 mg/kg/day or the same volume of 5% dextrose both in 4 divided doses. They concluded that NAC (40 mg/kg/day) that was commenced immediately after admission to ICU did not ameliorate the progression of MOF in this small cohort of patients. They inferred that routine prophylactic use of low-dose NAC in all critically ill patients does not provide positive protection.

Kalokerinos et al., (2005), discussed that endotoxin severely depletes antioxidants, in particular GSH and ascorbate. A depletion of antioxidants is directly associated with the severity of the reaction to endotoxin exposure. The deleterious effects of endotoxin are mediated by the immune system; the damage done to tissues is due to direct attack by radicals released from phagocytes. Ascorbate can overcome the radicals produced by the immune system if it is in sufficient and sustained concentration. Ascorbate also decreases or prevents endotoxin translocation from the gut, is directly bactericidal, and increases circulation and liver GSH concentrations.
Ascorbate also prevents a decline in the hepatic detoxifying enzymes responsible for endotoxin clearance. Ascorbate blocks the increase in iNOS expression responsible for increased NO production in sepsis. In sepsis, it is reasonable to suggest that patients should be supplemented continuously with oral and intravenous ascorbate.

Crimi et al., (2005), investigated whether intervention with antioxidant vitamins C and E in enteral feeding influenced oxidative stress and clinical outcome in critically ill patients. The study comprised of two-hundred sixteen patients who were expected to require at least 10 days of enteral feeding. One-hundred five patients received enteral feeding supplemented with antioxidants, and 111 control patients received an isocaloric formula. There was significantly reduced 28-day mortality after antioxidant intervention (45.7% in the antioxidant group and 67.5% in the regular-feeding group; p < 0.05).

Thimmulappa et al., (2006), investigated whether administration of an exogenous antioxidant could attenuate the augmented proinflammatory cascade in sepsis. Mice were pretreated with NAC (500 mg/kg body weight) and then challenged with nonlethal doses of LPS. Pretreatment of wild-type mice with NAC provided modest protection. Their findings suggested that the role of Nuclear factor-erythroid 2–related factor 2 (Nrf2) as a critical regulator of antioxidant gene expression, restoration of intracellular glutathione with NAC decreased LPS- and TNF-induced NF-κB activation and reduced LPS-induced lung injury in Nrf2 mice.

Controversies remain regarding the indications of antioxidant therapy, the combination of antioxidants, the doses, and the timing of supplementation. Berger and Chioléro, (2007), and in their review article summarized the current knowledge. They concluded that three antioxidant nutrients have demonstrated clinical benefits and reached level A evidence: a) selenium improves clinical outcome (infections, organ failure); b) glutamine reduces infectious complication in large-sized trials; and c) the association of eicosapentaenoic acid and micronutrients has significant anti-inflammatory effects.

There have been some controversy concerning whether antioxidants might attenuate oxidative damage and inflammation in humans after hypotension in the setting of critical illness. With this background Fraga et al., (2011), conducted a prospective, randomized, double-blinded, placebo-controlled study that included patients with hypotension. Patients were randomized to receive either NAC (50 mg/kg by 4 hours
followed by 100 mg/kg/d for 48 hours diluted in 5% glucose) and deferoxamine (DFx; at a single dose of 1000 mg diluted in 5% glucose) or placebo. On analysis of secondary outcomes, it was observed that creatinine levels at hospital discharge were lower in patients receiving NAC plus DFx when compared with placebo. They concluded that NAC plus DFx administration was able to decrease plasma markers of oxidative damage and creatinine levels at hospital discharge. 

Zhang et al., (2011), determined whether the antioxidant, NAC, could protect human kidney proximal tubule epithelial cells. However, although NAC is frequently utilized as a GSH precursor, the cytoprotection afforded by NAC in HK-2 cells was not a consequence of increased GSH levels. They speculated that NAC exerts its protective effect in part by directly scavenging ROS and in part via extracellular regulated kinase1/2 activation.