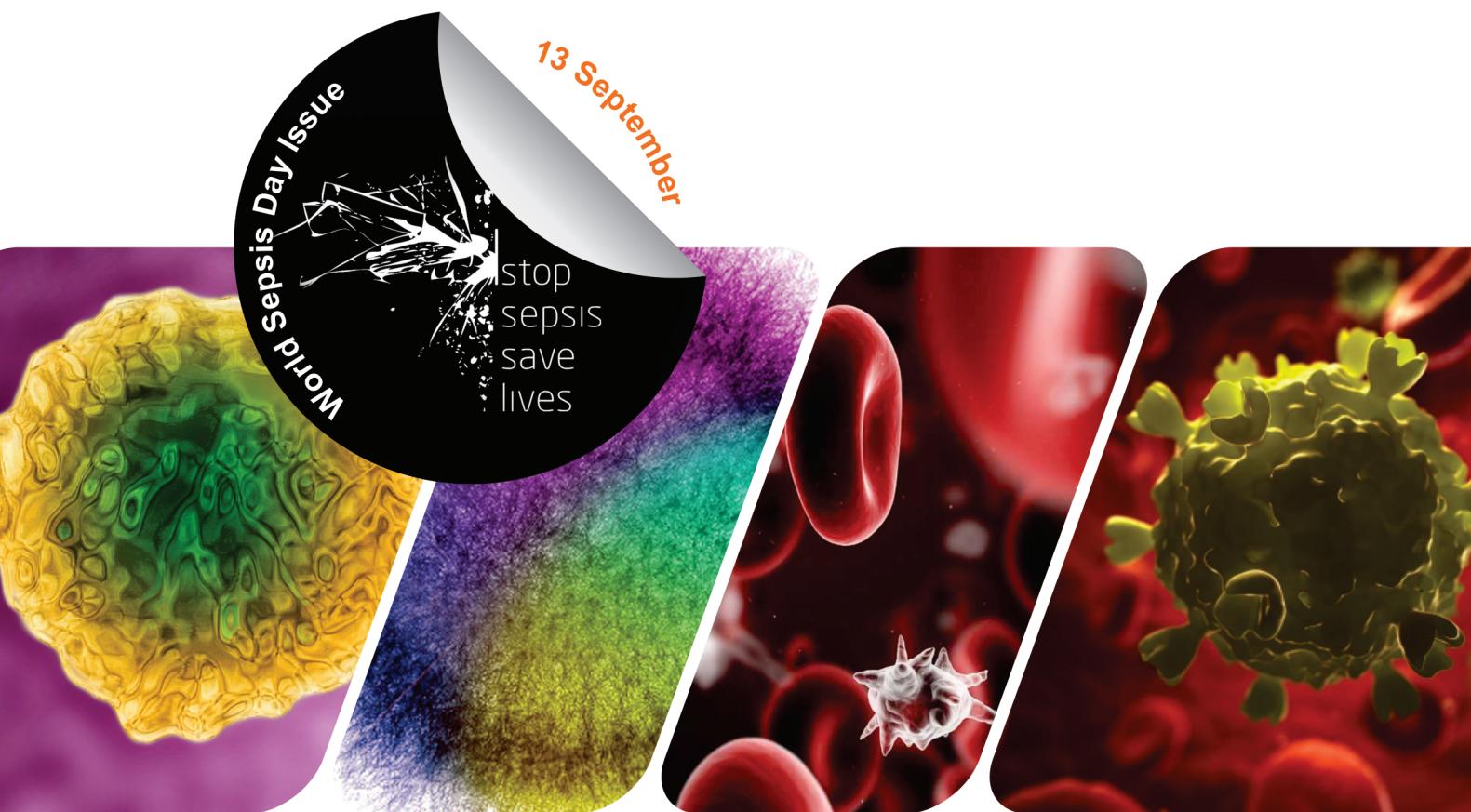


# SEEDS

## Managing Cytokine Storm



Issue 8

# The Story of our Ascent

**Setting sights on the peak**

**Extending the benefits of U-Tryp to 500 patients this World Sepsis Day**

**Reaching the 2<sup>nd</sup> summit**

**Over 12,000 patients treated with U-Tryp;**

**Accorded the Orphan Drug Status for AP in the European Union**

**Reaching the 1<sup>st</sup> summit**

**Trial published in international journal - Intensive Care Medicine**

**Strengthening the efforts to climb**

**U-Tryp gets stamp of approval for**

**Acute Pancreatitis management from DCGI**

**August  
2013**

**Getting the equipment ready**  
**U-Tryp Trial published in JAPI**

**September  
2012**

**Making preparations for the climb**  
**U-Tryp launched for tackling Sepsis**

**April  
2012**

**Studying the terrain**  
**U-Tryp gets stamp of approval from DCGI for Sepsis Management**

R  
**U-Tryp**  
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**PREFACE**

Over the last few decades, the burden of sepsis has increased relentlessly. It remains a critical problem with significant morbidity and mortality even in the modern era of critical care management. It is important to increase sepsis awareness by modulating communication strategies, and to no longer refer only to the infections that cause sepsis, rather consider sepsis as the major cause of death from infections. Therefore, World sepsis day is an initiative to accelerate the global action to highlight the devastating impact of sepsis.

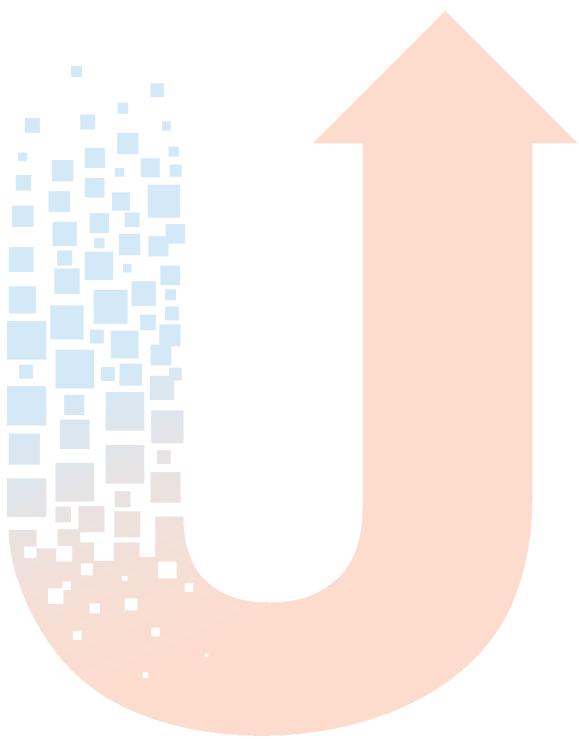
Evidences emerging from accrued clinical reports suggest an imbalance in the inflammatory network, resulting in sepsis. Cytokines are important pleiotropic regulators of the immune response, which have a crucial role in the complex pathophysiology underlying sepsis. They have both pro- and anti-inflammatory functions and are potentially effective against invading pathogens. On the other hand, cytokines may dysregulate the immune response and promote tissue-damaging inflammation. Excessive or uncontrolled release of proinflammatory cytokines is known as cytokine storm. An unchecked cytokine storm may eventually perpetuate multiorgan dysfunction, shock, and ultimately death in severe cases. Calming deranged immune response and restoring it back to normalcy would improve disease pathophysiology and its outcomes. Immunomodulators, such as protease inhibitors, have recently emerged as promising therapeutic agents. Ulinastatin is a protease (trypsin) inhibitor with anti-inflammatory immunomodulatory effects that help in attenuating the levels of proinflammatory cytokines. Furthermore, it has been showing encouraging results in both experimental studies and clinical trials involving sepsis and severe cases of malaria.

The eighth issue of SEEDS journal has been conceived with special emphasis on the above-mentioned subjects. The article on sepsis management gives an overview of the pathogenesis and encompasses several bundles involved in the treatment of sepsis. Secondly, a review on the dynamics of a cytokine storm has been highlighted. This is followed by a review on the causes, differential diagnosis and management of viral encephalitis. Lastly, a comprehensive discussion on the pathogenesis, clinical manifestations, diagnostic approach and management of malaria including the potential role of ulinastatin as an emerging therapeutic strategy has also been incorporated.

We hope that the information provided in this journal will provide our readers with all the relevant and recent information they are looking for.

Thanks & regards

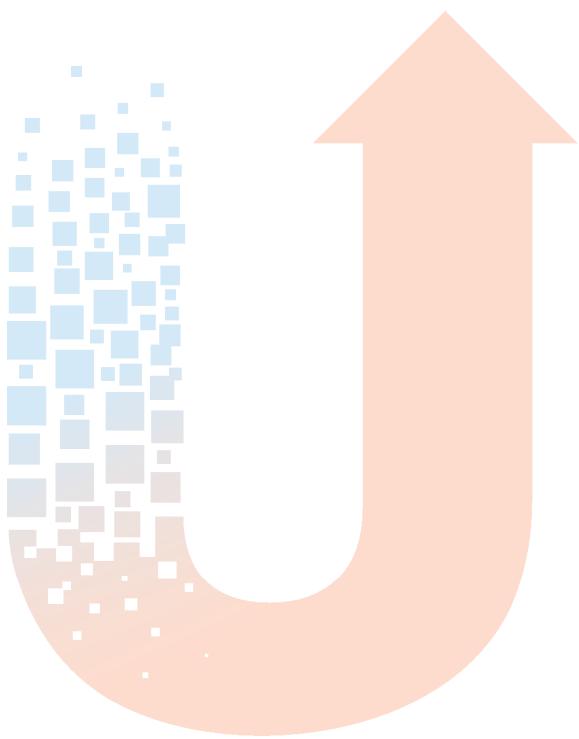
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# The sepsis resuscitation and management bundles

## Global burden of sepsis

Sepsis is a condition that results from a harmful or damaging host response to infection. It can lead to shock, multiple organ failure, and death, especially if not recognized early and treated promptly.<sup>1</sup> Wide prevalence of sepsis has been reported across the globe in the past, with the enormity of its impact witnessed in both the developed and developing countries. In developing countries, it accounts for 60-80% of all deaths. It kills more than 6 million infants and young children, and 1,00,000 new mothers every year. It remains the primary cause of death from infection, despite advances in modern medicine like vaccines, antibiotics, and intensive care.<sup>2</sup> Over the last decade, hospitalizations for sepsis have increased by more than two folds.<sup>3</sup> Evidence based studies have revealed that 20-40% of sepsis patients requiring intensive care treatment developed sepsis outside the hospital.<sup>4</sup>

## Pathogenesis of sepsis: An overview

The body's natural defense mechanism prevents the pathogens from spreading into the circulatory system. Local infection in a patient causes local inflammation. This leads to expansion of the blood vessels around the center of infection. These blood vessels become more permeable, thus increasing the blood supply. Additionally, the blood circulates at a slower pace to allow leukocytes and semiochemicals to penetrate the vascular walls into the tissues to fight the pathogens. The blood in the micro-vessels surrounding the infection coagulates. The hallmarks of inflammation including, increased temperature, redness, pain, and swelling, appear around the center of infection.<sup>5</sup>

Failure of these local defense mechanisms results

in entry of pathogens into the circulatory system. This is followed by the spread of the natural inflammatory response, which in turn, damages organs and tissues that have not been invaded directly by pathogens.<sup>5</sup>

Furthermore, individuals with compromised immune systems are more likely to develop sepsis. This is due to the fact that local defense mechanisms against the invasion of pathogens into the blood stream, in these individuals are weakened, and their immune response to invasion is often too weak. In severe cases, hypotension occurs, condition of the heart worsens, oxygen supply to the blood via the lungs deteriorates, the oxygen supply to organs and tissues is choked, the kidneys no longer produce urine, and the patient's mental status is gravely impaired.<sup>5</sup>

The three stages of sepsis are:

### Stage 1:

A local infection overpowers the body's local defense mechanisms. Pathogens and toxins, then, enter the circulatory system. This leads to a general inflammatory response called systemic inflammatory response syndrome (SIRS). The various criteria for SIRS are mentioned in box 1.<sup>5,6</sup>

### Stage 2:

Initiation of organ function deterioration occurs. The functions of organs may completely fail.<sup>5</sup>

### Stage 3:

Several organs stop functioning sequentially or simultaneously, and cardio-circulatory failure leads to hypotension, resulting in septic shock.<sup>5</sup> Figure 1 comprehensively depicts the three stages of sepsis.

**Box 1: Criteria for the systemic inflammatory response syndrome, adapted from the American College of Chest Physicians/Society Critical Care Medicine Consensus Conference**

**Two or more of the following are required:**

- 1) Body temperature  $>38^{\circ}\text{C}$  or  $<36^{\circ}\text{C}$
- 2) Heart rate  $>90$  beats per minute
- 3) Respiratory rate  $>20$  breaths per minute or arterial CO<sub>2</sub> tension  $<32$  mmHg or a need for mechanical ventilation
- 4) White blood count greater than  $12,000 \text{ mm}^{-3}$  or  $<4000/\text{mm}^3$  or  $>10\%$  immature forms

**Sources:**

1. Sepsis is the most common pathway following to death following an infection. It can be reduced. Available at: <http://www.world-sepsis-day.org/?MET=SHOWCONTAINER&vPRIMNAVISELECT=3&vSEKNAVISELECT=2&vCONTAINERID=>. Accessed on: 28.7.2014.
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## Diagnostic approach to sepsis

Lack of adequate diagnostic aids (clinical symptoms and laboratory signs) often leads to delayed diagnosis of sepsis, particularly in children, in whom the signs and symptoms may be subtle. This increases the rate of deterioration. Immediate initiation of interventions, including antimicrobials and intravenous fluids, may help in decreasing the rate of mortality by half.<sup>7</sup> Early sepsis treatment is cost effective, and reduces the number of hospital and critical care bed days for patients.<sup>3</sup> The diagnostic criteria for sepsis and, severe sepsis, advised by

the Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock: 2012 is mentioned in tables 1 and 2, respectively.<sup>8</sup>

Diagnostic criteria for sepsis in the pediatric population include signs and symptoms of inflammation, and infection with hyper- or hypothermia (rectal temperature  $>38.5^{\circ}$  or  $<35^{\circ}\text{C}$ ), tachycardia (may be absent in hypothermic patients), and at least one of the following indications of altered organ function:

- Altered mental status
- Hypoxemia
- Increased serum lactate level or
- Bounding pulses.

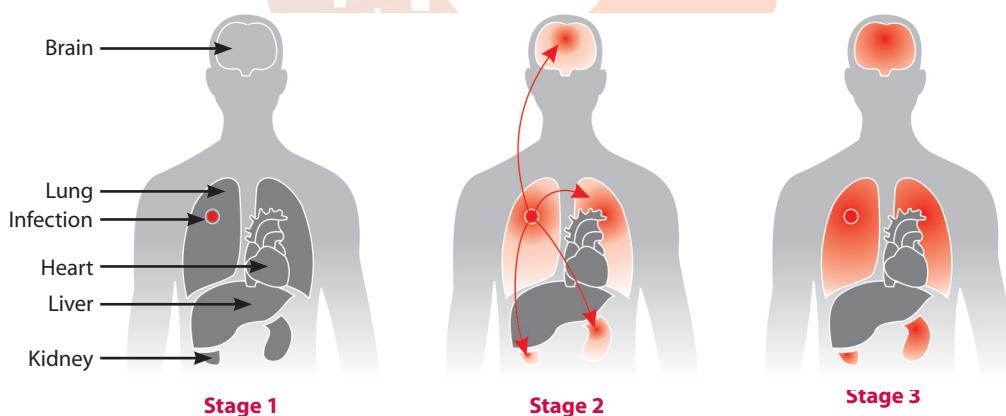
## How to prevent sepsis?

The development of sepsis occurs due to an infection, most often caused by bacteria, followed by fungi or protozoa. Therefore, preventing infection can help prevent sepsis. Following measures may prove to be beneficial in preventing infections:

### Vaccination

Infants, children and elderly are more susceptible to infections, particularly to pneumococcus bacteria. This can lead to pneumonia, middle ear infections, sinusitis, meningitis and ultimately sepsis. The use of vaccines may help in developing immunity to major pneumococcus pathogens. Vaccinating small children leads to a greater

**Figure 1: Stages of sepsis**



**Source:** Sepsis is the most common pathway to death following an infection. It can be reduced. Available at: <http://www.world-sepsis-day.org/?MET=SHOWCONTAINER&vPRIMNAVISELECT=3&vSEKNAVISELECT=2&vCONTAINERID=>. Accessed on 28.7.2014.

**Table 1: Diagnostic Criteria for Sepsis**

- General variables
  - » Fever ( $> 38.3^{\circ}\text{C}$ )
  - » Hypothermia (core temperature  $< 36^{\circ}\text{C}$ )
  - » Heart rate  $> 90/\text{min}^{-1}$  or more than two SD above the normal value for age
  - » Tachypnea
  - » Altered mental status
  - » Significant edema or positive fluid balance ( $> 20 \text{ mL/kg over } 24 \text{ hr}$ )
  - » Hyperglycemia (plasma glucose  $> 140 \text{ mg/dL}$  or  $7.7 \text{ mmol/L}$  in the absence of diabetes)
- Inflammatory variables
  - » Leukocytosis (WBC count  $> 12,000 \mu\text{L}^{-1}$ )
  - » Leukopenia (WBC count  $< 4000 \mu\text{L}^{-1}$ )
  - » Normal WBC count with greater than 10% immature forms
  - » Plasma C-reactive protein more than two SD above the normal value
  - » Plasma procalcitonin more than two SD above the normal value
- Hemodynamic variables
  - » Arterial hypotension (SBP  $< 90 \text{ mmHg}$ , MAP  $< 70 \text{ mmHg}$ , or SBP decrease  $> 40 \text{ mmHg}$  in adults or less than two SD below normal value for age)
- Organ dysfunction variables
  - » Arterial hypoxemia ( $\text{PaO}_2/\text{FiO}_2 < 300$ )
  - » Acute oliguria (urine output  $< 0.5 \text{ mL/kg/hr}$  for at least 2 hrs despite adequate fluid resuscitation)
  - » Creatinine increase  $> 0.5 \text{ mg/dL}$  or  $44.2 \mu\text{mol/L}$
  - » Coagulation abnormalities (INR  $> 1.5$  or aPTT  $> 60 \text{ s}$ )
  - » Ileus (absent bowel sounds)
  - » Thrombocytopenia (platelet count  $< 100,000 \mu\text{L}^{-1}$ )
  - » Hyperbilirubinemia (plasma total bilirubin  $> 4 \text{ mg/dL}$  or  $70 \mu\text{mol/L}$ )
- Tissue perfusion variables
  - » Hyperlactatemia ( $> 1 \text{ mmol/L}$ )
  - » Decreased capillary refill or mottling

WBC: White blood cell; SBP: Systolic blood pressure; MAP: Mean arterial pressure; INR: International Normalized Ratio; aPTT: Activated partial thromboplastin time

**Source:** Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock: 2012. Available at: <http://www.world-sepsis-day.org/CONTENTPIC/SSC-Guidelines.pdf>. Accessed on: 28.7.2014.

mechanism known as “herd immunity”, disrupting chains of infection and resulting in lesser pneumococcus infections even among those not vaccinated. Furthermore, vaccinations against pneumococcus, meningococcus and hemophilus bacteria, are peculiarly important for patients who have lost their spleen or who were born without a fully functioning spleen. These individuals are at a greater

**Table 2: Diagnostic criteria for severe sepsis (Sepsis-induced tissue hypoperfusion or organ dysfunction)**

- Sepsis-induced hypotension
- Lactate above upper limits
- Urine output  $< 0.5 \text{ mL/kg/hr}$  for more than 2 hrs despite adequate fluid resuscitation
- Acute lung injury with  $\text{PaO}_2/\text{FiO}_2 < 250$  in the absence of pneumonia as infection source
- Acute lung injury with  $\text{PaO}_2/\text{FiO}_2 < 200$  in the presence of pneumonia as infection source
- Creatinine  $> 2 \text{ mg/dL}$  ( $176.8 \mu\text{mol/L}$ )
- Bilirubin  $> 2 \text{ mg/dL}$  ( $34.2 \mu\text{mol/L}$ )
- Platelet count  $< 100,000 \mu\text{L}$
- Coagulopathy (INR  $> 1.5$ )

**Source:** Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock: 2012. Available at: <http://www.world-sepsis-day.org/CONTENTPIC/SSC-Guidelines.pdf>. Accessed on: 28.7.2014.

risk of developing sepsis, and this risk remains throughout their lives. Unfortunately, most of these people have not been properly vaccinated against the bacteria that can trigger sepsis. Additionally, lack of awareness of the associated risk is still a major issue to be dealt with.<sup>5</sup>

### The indiscriminate use of antibiotics must be discontinued

Another strategy to reduce mortality rate resulting from sepsis is prevention of bacterial resistance to antibiotics. Over the last few years, excessive use of antibiotics in outpatient care has considerably increased antibiotic-resistant bacteria, particularly bacteria on the skin and fecal bacteria. The danger posed by bacteria on the skin (MRSA) is vastly overestimated, as they can be treated quite easily in most cases. Thus, judicious use of antibiotics should be encouraged.

### Sanitation

Environmental sanitation is a major cause of concern. Insufficient hygiene conditions in resource-poor areas for giving birth, treating wounds, and in healthcare facilities in general, are the fundamental issues underlying infectious diseases. Unsanitary facilities or contaminated water cause severe infections in the digestive system, which often leads to sepsis. Therefore, hand hygiene and good general hygiene practices, clean deliveries, improvements in sanitation and nutrition, delivery of clean water, and vaccination programs for individuals at risk in resource-poor areas should be promoted.<sup>5</sup>



## Sepsis is a medical emergency

Sepsis is a deadly and potentially preventable complication. A better understanding of sepsis may help in providing appropriate intervention strategies, thus saving patient's life. Emergency medical treatment is the only hope of survival. Management relies on the early identification and treatment of the underlying causative

infection. Immunomodulatory therapies may be beneficial in enhancing or controlling the body's immune response, as needed. In addition to antimicrobial treatments, these therapies may help to increase a patient's chances of survival in the future. Recommendations by the Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock: 2012 are mentioned in table 3.<sup>8</sup>

**Table 3: Recommendations by the Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock**

### Initial resuscitation

- Protocolized, quantitative resuscitation of patients with sepsis-induced tissue hypoperfusion (hypotension persisting after initial fluid challenge or blood lactate concentration  $\geq 4$  mmol/L). Goals during the first 6 hours of resuscitation include:
  - » Central venous pressure 8–12 mmHg
  - » Mean arterial pressure (MAP)  $\geq 65$  mmHg
  - » Urine output  $\geq 0.5$  mL/kg/hr
  - » Central venous (superior vena cava) or mixed venous oxygen saturation 70% or 65%, respectively
- In patients with elevated lactate levels, target resuscitation to normalize lactate

### Screening for sepsis and performance improvement

- Routine screening of potentially infected seriously ill patients for severe sepsis to allow early implementation of therapy
- Hospital-based performance improvement efforts in severe sepsis

### Diagnosis

- Cultures as clinically appropriate before antimicrobial therapy if no significant delay ( $> 45$  mins) in the start of antimicrobial(s). At least 2 sets of blood cultures (both aerobic and anaerobic bottles) should be obtained before antimicrobial therapy with at least 1 drawn percutaneously and 1 drawn through each vascular access device, unless the device was recently ( $< 48$  hrs) inserted
- Use of 1,3 beta-D-glucan assay, mannan and anti-mannan antibody assays, if available and invasive candidiasis is in differential diagnosis of cause of infection
- Imaging studies performed promptly to confirm a potential source of infection

### Antimicrobial therapy

- Administration of effective intravenous antimicrobials within the first hour of recognition of septic shock and severe sepsis without septic shock as the goal of therapy
- Initial empiric anti-infective therapy of one or more drugs that have activity against all likely pathogens (bacterial/fungal/viral) and that penetrate in adequate concentrations into tissues presumed to be the source of sepsis
- Antimicrobial regimen should be reassessed daily for potential de-escalation
- Use of low procalcitonin levels or similar biomarkers to assist the clinician in the discontinuation of empiric antibiotics in patients who initially appeared septic, but have no subsequent evidence of infection
- Combination empirical therapy for neutropenic patients with severe sepsis and for patients with difficult-to-treat, multi drug resistant bacterial pathogens such as *acinetobacter* and *pseudomonas* species. For patients with severe infections associated with respiratory failure and septic shock, combination therapy with an extended spectrum beta-lactam and either an aminoglycoside or a fluoroquinolone is for *P. aeruginosa* bacteremia. A combination of beta-lactam and macrolide for patients with septic shock from bacteraemic *Streptococcus pneumoniae* infections
- Empiric combination therapy should not be administered for more than 3–5 days. De-escalation to the most appropriate single therapy should be performed as soon as the susceptibility profile is known
- Duration of therapy typically 7–10 days; longer courses may be appropriate in patients who have a slow clinical response, undrainable foci of infection, bacteremia with *S. aureus*; some fungal and viral infections or immunologic deficiencies, including neutropenia
- Antiviral therapy initiated as early as possible in patients with severe sepsis or septic shock of viral origin
- Antimicrobial agents should not be used in patients with severe inflammatory states, determined to be of noninfectious cause



### Fluid therapy of severe sepsis

- Crystalloids as the initial fluid of choice in the resuscitation of severe sepsis and septic shock
- Albumin in the fluid resuscitation of severe sepsis and septic shock when patients require substantial amounts of crystalloids
- Initial fluid challenge in patients with sepsis-induced tissue hypoperfusion with suspicion of hypovolemia to achieve a minimum of 30 mL/kg of crystalloids (a portion of this may be albumin equivalent). More rapid administration and greater amounts of fluid may be needed in some patients

### Vasopressors

- Initially target a MAP of 65 mmHg, norepinephrine (NE), being the first choice vasopressor
- Epinephrine (added to and potentially substituted for NE) when an additional agent is needed to maintain adequate blood pressure
- Vasopressin 0.03 units/minute can be added to NE with intent of either raising MAP or decreasing NE dosage
- Low dose vasopressin is not recommended as the single initial vasopressor for treatment of sepsis-induced hypotension and vasopressin doses higher than 0.03-0.04 units/minute should be reserved for salvage therapy (failure to achieve adequate MAP with other vasopressor agents)
- Dopamine as an alternative vasopressor agent to NE only in highly selected patients (patients with low risk of tachyarrhythmias and absolute or relative bradycardia); low dose dopamine should not be used for renal protection
- Phenylephrine is not recommended in the treatment of septic shock except in circumstances where
  - » Norepinephrine is associated with serious arrhythmias
  - » Cardiac output is known to be high and blood pressure persistently low or
  - » As salvage therapy when combined inotrope/vasopressor drugs and low dose vasopressin have failed to achieve MAP target

### Source control

- A specific anatomical diagnosis of infection requiring consideration for emergent source control be sought and diagnosed or excluded as rapidly as possible, and intervention be undertaken for source control within the first 12 hr after the diagnosis is made, if feasible
- When infected peripancreatic necrosis is identified as a potential source of infection, definitive intervention is best delayed until adequate demarcation of viable and nonviable tissues has occurred
- When source control in a severely septic patient is required, the effective intervention associated with the least physiologic insult should be used, such as percutaneous rather than surgical drainage of an abscess
- If intravascular access devices are a possible source of severe sepsis or septic shock, they should be removed promptly after other vascular access has been established

### Infection prevention

- Selective oral decontamination and selective digestive decontamination should be introduced and investigated as a method to reduce the incidence of ventilator-associated pneumonia
- Oral chlorhexidine gluconate be used as a form of oropharyngeal decontamination to reduce the risk of ventilator-associated pneumonia in ICU patients with severe sepsis

**Source:** Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock: 2012. Available at: <http://www.world-sepsis-day.org/CONTENTPIC/SSC-Guidelines.pdf>. Accessed on: 28.7.2014.

## Surviving sepsis

Sepsis is a life threatening condition. After a patient has survived the acute phase of sepsis, various serious symptoms often remain. The extent of these complications depends on the severity of sepsis and the length of stay in the intensive care unit and in the hospital. These complications often persist for years after the actual sepsis attack. The lack of specific, standardized rehabilitation programs for sepsis patients, slows down or hinders full recovery. These medical conditions often have negative impact on the survivor's life and lifestyle.<sup>9</sup>

## Conclusion

Sepsis can be considered a race to the death between invading pathogens and the host immune response and the pathogens seek an advantage by disabling selected aspects of host defenses. Severe sepsis and septic shock are currently among the most common causes of morbidity and mortality in intensive care, and their incidences have increased relentlessly, over the past decade. Adhering to simple preventative measures may help in reducing infection, thus preventing the development of sepsis. Furthermore, early recognition and effective treatment

## A case study of a patient who survived sepsis

The patient's illness started with fever and chills. The fever continued to rise during the night to over 104°F. The next morning, he had blue patches on his face and arms, which were diagnosed as dermorrhagia. He was admitted to the intensive care unit of a hospital, where a patient had died four months earlier of pneumococcus sepsis. He was suspected with the same and was transferred to another hospital, after inducing an artificial coma. By the time he reached there, his liver, lungs, and kidneys had stopped functioning, and he was put on dialysis.

The patient had lost his spleen at the age of seven, following a car accident. Thus, his body was lacking a critical immune function. Investigations confirmed that he had contracted pneumococci. He lost 15 kg and had multiple organ failure. Doctors had to operate his brain due to raised intracranial pressure. Also, his two fingers were amputated. All while the patient was in coma. After five long weeks, he was woken up as his organs were relatively stabilized. He was unable to move, and his body was wracked with pain. His muscles had completely atrophied. In a rehabilitation centre, he had to learn everything again including activities like eating, drinking, washing, brushing, getting dressed, and walking.

The patient regretted of not getting himself vaccinated against pneumococci, meningococcal, and *hemophilus influenza*, which would probably have prevented the development of sepsis.

**Source:** Sepsis is the most common pathway following to death following an infection. It can be reduced. Available at: [http://www.world-sepsis-day.org/?MET=SHOWCONTAINER&v\\_PRIMNAVISELECT=3&vSEKNAVISELECT=2&vCONTAINERID=](http://www.world-sepsis-day.org/?MET=SHOWCONTAINER&v_PRIMNAVISELECT=3&vSEKNAVISELECT=2&vCONTAINERID=). Accessed on: 28.7.2014.



may diminish the impact of this devastating disease. A greater awareness among clinicians and across the society is crucial for future success. To that end, the World Sepsis Day is a welcome and wonderful initiative.

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# Dynamics of a cytokine storm

## Introduction

Cytokines are signaling peptides, proteins, or glycoproteins, involved in either augmentation or suppression of inflammation that occurs in response to pathogens, non-self molecules or toxins.<sup>1</sup> Several cells release cytokines including immune, epithelial, endothelial, and smooth muscle cells.<sup>2</sup> Cytokines allow context-dependent communication within the body.<sup>3,4</sup> Any impairment in cytokine production leads to uncontrolled inflammation within tissues and key organs, termed as a "cytokine storm" or hypercytokinemia.<sup>1</sup> Association of cytokine storms with sepsis and septic shock, influenza, acute respiratory distress is well established. In addition, cytokine storms occur in relation with host response to blood transfusion or bone marrow transplantation, and toxic response to medication.<sup>5,6</sup> Cytokine response occurs through a complex network, involving a series of overlapping networks, each with a degree of redundancy and with alternate pathways. This combination of overlap and redundancy exert significant implications to determine vital factors that contribute in the cytokine response to infection. Moreover, it also helps in targeting specific cytokines for therapeutic interventions.<sup>7</sup>

## Cytokines associated with the cytokine storm

The cytokine family of proteins forms an integral part of the signaling network between cells. It plays a pivotal role in generating and regulating the immune system. Specific cytokines exhibit autocrine, paracrine, and/or endocrine activity and, through receptor binding, stimulate various responses, depending upon the cytokine and the target cell.<sup>7</sup> Cytokines may have multiple and oftentimes unrelated functions that depend on the target cell or on the presence or absence of other cytokines. Some cytokines have limited sequence similarity and engage distinct receptors yet transduce signals through common intracellular pathways.<sup>8</sup> The major types and actions of cytokines are mentioned in table 1.<sup>7</sup>

## Interferons

The interferons (IFNs) are a group of secreted cytokines that play a key role in innate immunity to viruses and other microbial pathogens. They are categorized into three major types (I, II, III), according to their receptor specificity.<sup>9,10</sup> Type I IFNs (IFN-alpha and IFN-beta) signal through a heterodimeric receptor complex, IFNAR1/

**Table 1: Major types and actions of cytokines**

| Type                       | Actions  |
|----------------------------|--|
| Interferons                | Regulation of innate immunity, activation of antiviral properties, antiproliferative effects |
| Interleukins               | Growth and differentiation of leukocytes, many are proinflammatory                           |
| Chemokines                 | Control of chemotaxis, leukocyte recruitment, many are proinflammatory                       |
| Colony-stimulating factors | Stimulation of hematopoietic progenitor cell proliferation and differentiation               |
| Tumor necrosis factor      | Proinflammatory, activates cytotoxic T lymphocytes   |

**Source:** Jennifer R. Tisoncik JR, Korth MJ, Cameron P. Simmons CP, Farrar J, Martin TR, Katze MG. Into the Eye of the Cytokine Storm. *Microbiol Mol Biol Rev.* 2012;76(1):16-32.

IFNAR2 while type II IFN (IFN-gamma) signals through IFN-gammaR1/IFN-gammaR2. Lambda IFN is another category of IFN with antiviral properties, and has shown benefits in providing protection against influenza A virus in experimental models.<sup>11,12</sup> Although IFN-gamma 1, -gamma 2, and -gamma 3 [also referred to as interleukin-29 (IL-29), IL-28a, and IL-28b, respectively] bind receptor complex IL-28R/IL-10R beta however, they demonstrate functional similarity to type I IFNs, as both type I and type II IFNs transduce signals through the Jak-STAT signaling pathway. Receptor binding leads to the initiation of downstream signaling cascades which ultimately activates transcription factors and induces several IFN-stimulated genes. These genes, in turn, encode protein products with antiviral, antiproliferative, or immunomodulatory properties (figure 1). These effects contribute to the therapeutic use of IFNs in the treatment of viral diseases such as hepatitis C and hepatitis B, certain types of leukemia and lymphoma, and multiple sclerosis.<sup>13,14</sup>

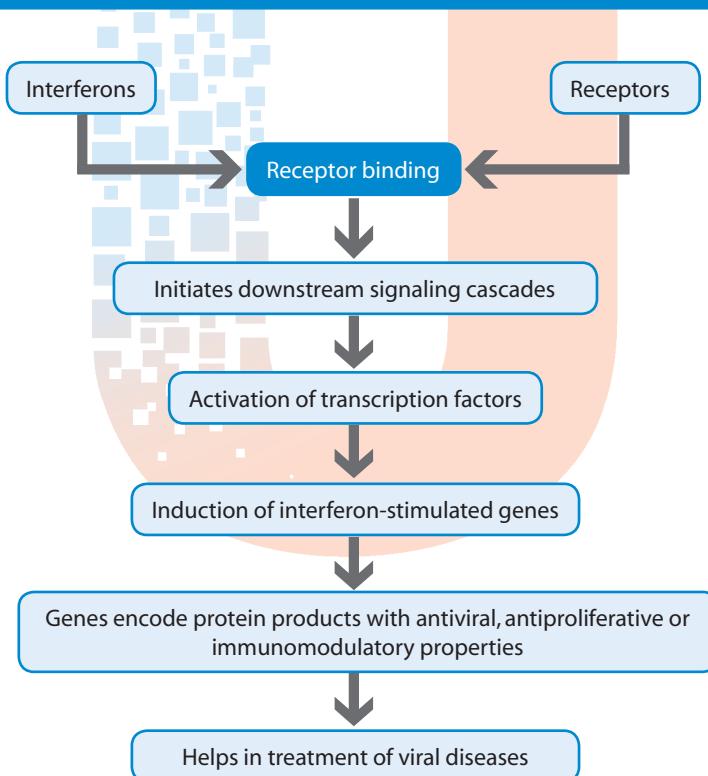
## Interleukins

Unlike INFs, the interleukins constitute a diverse family of immune system regulators, synthesized by many cell types. They are primarily involved in immune cell differentiation and activation, and may be either pro- or anti-inflammatory cytokines.<sup>15</sup> IL-1 alpha and IL-1 beta are proinflammatory cytokines that mediate the host response to infection through both direct and indirect mechanisms.<sup>16</sup> Biological functions of these cytokines are shown in figure 2.<sup>17</sup> Various local and systemic effects (predominantly proinflammatory) occur as a consequence of acute-phase response to infection, which help in eradicating viruses.<sup>18</sup>

## Chemokines

Chemokines is the largest group of cytokines having 44 members that bind to one or more of 21 G-protein-coupled receptors.<sup>19</sup> They are further classified into four types (CXC, CC, C, and CX<sub>3</sub>C), depending upon the spacing

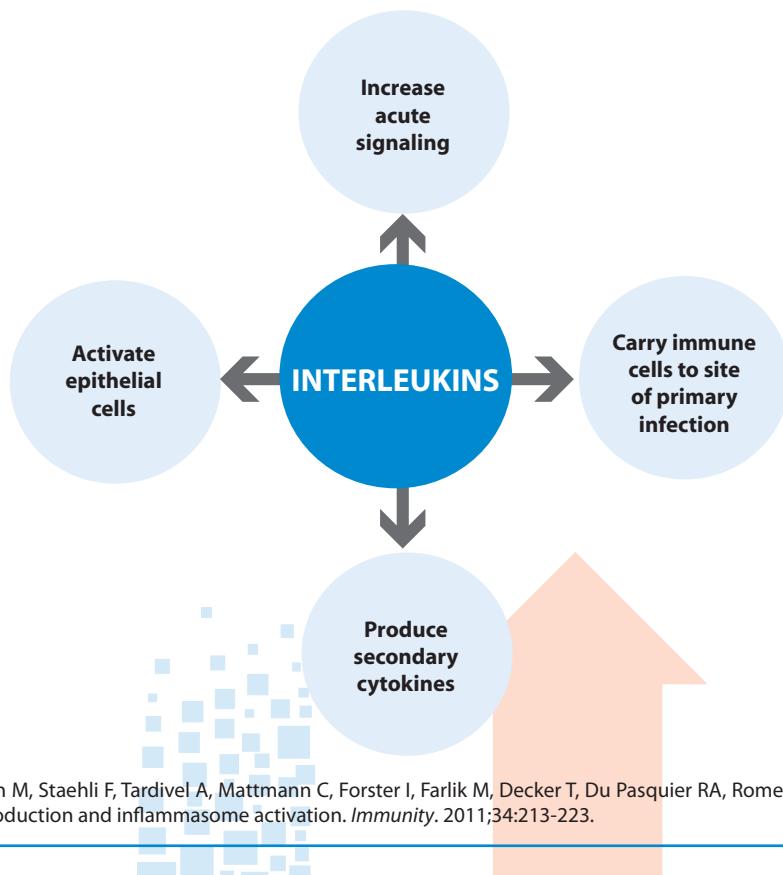
**Figure 1: Mechanism of action of interferons (Jak-STAT signaling pathway)**



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1. Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, Foster GR, Stark GR. Interferons at age 50: past, current and future impact on biomedicine. *Nat Rev Drug Discov.* 2007;6(12):975-90.
2. Friedman RM. Clinical uses of interferons. *Br J Clin Pharmacol.* 2008;65(2):158-62.

Figure 2. Biological functions of interleukins



**Source:** Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Forster I, Farlik M, Decker T, Du Pasquier RA, Romero P, Tschopp J. Type I interferon inhibits interleukin-1 production and inflammasome activation. *Immunity*. 2011;34:213-223.

of their first two cysteine residues. The primary function of chemokines is to control the migration of cells, specifically those of the immune system.<sup>20</sup> Most of the chemokines are considered to be proinflammatory, and are released by a variety of cells in response to infection. This in turn results in recruitment of immune system cells including, neutrophils, monocytes/macrophages, and lymphocytes to the site of infection.<sup>21</sup>

### Colony-stimulating factors

Colony-stimulating factors (CSFs) including granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), and granulocyte colony-stimulating factor (G-CSF) are involved in stimulation of hematopoietic progenitor cell proliferation and differentiation. Evidence based observations suggest the association of CSFs with inflammation. They may be a part of a mutually dependent proinflammatory cytokine network that includes IL-1 and tumor necrosis factor (TNF).<sup>22</sup>

### Tumor necrosis factor

Tumor necrosis factor proteins consist of 19 members that signal through 29 receptors. TNF is expressed by a variety of immune cells, and its primary receptor, TNFR1, appears to be expressed by all cell types, ensuring widespread effects of this cytokine. TNF plays a vital role in acute viral diseases, including those caused by influenza virus, dengue virus, and Ebola virus.<sup>23</sup> Excessive production of TNF is associated with several chronic inflammatory and autoimmune diseases.<sup>24</sup>

### Cytokine dynamics

The immune response to an infection is intriguingly complex and dynamic in nature. Despite extensive research, the precise knowledge on the immune response remains obscure. Several studies have been conducted to unravel cytokine dynamics during infections.<sup>7</sup> The investigators of a study showed that TNF is capable of promoting IL-1 generation, which causes alterations in endothelial cell physiology within the local microenvironment.<sup>25</sup> In addition, it can also exert broad



systemic effects beyond the site of infection. A study involving experimental models with severe *Pseudomonas* pneumonia and systemic sepsis provided definitive evidence that TNF instilled into the lungs could pass into the systemic circulation, providing direct communication between the lungs and the bloodstream.<sup>26</sup>

Studies in the past employed intermittent sampling from one compartment (typically the peripheral blood), although many critical responses are likely to be far more localized in tissue. For instance, in severe infections affecting the deep tissues of the respiratory tract, it is probable that it is the immunological cascade that takes place directly in the deep tissues that is crucial to immunopathology rather than what can be measured as a "spillover" in the peripheral blood.<sup>27</sup> Considering the compartmentalization of tissue-specific microenvironments is also of paramount importance. For instance, respiratory epithelium and alveolar macrophages in the lung normally maintain homeostasis by limiting activation of innate immunity in airways and alveolar spaces.<sup>28</sup> Similar compartmentalization is also important in central nervous system infections such as bacterial and tuberculous meningitis, encephalitis, fungal infections, and in infections such as dengue, in which the clinical syndrome is dominated by capillary permeability and plasma leakage.<sup>7</sup>

## Regulation of pro and anti-inflammatory cytokines

Production of the proinflammatory cytokines IL-1 and TNF-alpha produced by monocytes and macrophages, have been attributed to many infectious and idiopathic diseases. These key proinflammatory cytokines, in turn, stimulate the production of additional cytokines which, ultimately results in tissue pathology. A major deactivator of activated, cytokine-producing monocytes and macrophages is the anti-inflammatory cytokine IL-10. The interactions between these three cytokines are pivotal in terms of health and pathology however, dynamics of these interactions remain elusive to the investigators. A study modelled the autocrine interactions of TNF-alpha, IL-1 and IL-10 with monocytes. The model constructed was a six-dimensional, continuous-time dynamical system, with free IL-1 and IL-10 concentrations in the cell's vicinity, and the proportions of bound and free IL-1 and IL-10 cell-surface receptors, which transduced the cell's response to stimulation, as the state variables. The monocyte was assumed to be initially in a quiescent state, and it was stimulated to produce IL-1 by an external stimulus such as exposure to TNF-alpha or lipopolysaccharide.

This, in turn, invoked an autocrine IL-1 response, and also induced the production of the anti-inflammatory cytokine IL-10, which acted to downregulate IL-1 production. These responses were mediated by specific cell-surface receptors, the concentrations of which were also subjected to stimulated upregulation.<sup>29</sup>

In lungs, the intensity of the inflammatory response reflects a balance between proinflammatory cytokines (TNF and IL-1 beta) and their associated soluble receptors or inhibitors (TNFR1, TNFR2, and IL-1RA), which inhibit the activity of these inflammatory cytokines in the aqueous phase of alveolar fluid. Lung inflammation can be ameliorated by regulating the activation of specific cell types. For instance, CD200R expression on alveolar macrophages helps in resolving lung inflammation during influenza virus infection by restraining macrophage activity. Negative regulators, such as IL-1 receptor-associated kinase (IRAK-M), suppressor of cytokine signaling 1 (SOCS1), phosphoinositide-3-OH kinase (PI3K), Toll-interacting protein (TOLLIP), and zinc finger protein A20, also help in maintaining innate immune processes by preventing aberrant TLR activation.<sup>7</sup>

Another mechanism involved in reduction of inflammation is production of anti-inflammatory cytokines, particularly IL-10 by macrophages and certain types of T cells (Th2 and regulatory T cells) and B cells. It is also noteworthy that IL-10 (anti-inflammatory cytokine) has been demonstrated to play a role in fibrosis. Evidence based observations showed that increased IL-10 expression was reported to induce collagen production and fibrocyte recruitment into the lung. Additionally, interactions between IL-6 and its soluble receptor enhanced the activity of IL-6 on target cells. This provided a mechanism to increase the activity of TNF and IL-1 beta when the concentrations of soluble TNF receptors and IL-1RA are very high. As described above, the balance of pro- and anti-inflammatory mechanisms is critical to maintain immune homeostasis. Therefore, it appears plausible that absence or aberrant regulation of one or more of these regulatory mechanisms may contribute towards a cytokine storm.<sup>7</sup>

## Cytokine storm pathology

Cytokine storm is associated with inflammation which initiates at a local site. Systemic circulation spreads it throughout the body. Rubor (redness), tumor (swelling or edema), calor (heat), dolor (pain), and "functio laesa" (loss of function) are the hallmarks of acute inflammation. When localized in skin or other tissue, these responses increase blood flow, enable vascular leukocytes and



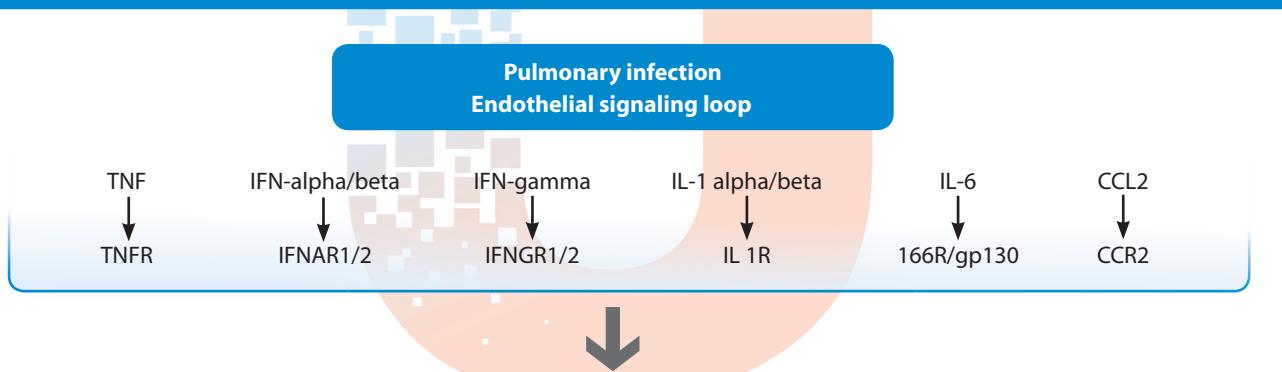
plasma proteins to reach extravascular sites of injury, increase local temperatures, and generate pain, thereby warning the host of the local responses. These responses often occur at the expense of local organ function, specifically when tissue edema raises extravascular pressures and reduces tissue perfusion. As soon as the inflammation begins, activation of compensatory repair processes occurs. Repair process may completely restore tissue and organ function or result in persistent organ dysfunction due to severe inflammation.<sup>7</sup>

The cytokine storm is best exemplified by severe lung infections. Local inflammation in lung infections spreads into the systemic circulation, resulting in development of systemic sepsis. This is associated with persistent hypotension, hyper- or hypothermia, leukocytosis or leukopenia, and often thrombocytopenia.<sup>30</sup> Sepsis syndrome may be caused by viral, bacterial, or fungal pulmonary infections. Differentiation of these etiological agents on clinical grounds is a perplexing issue. Persistent tissue damage in the lungs without severe microbial infection is also associated with a cytokine storm. Figure 3 comprehensively depicts the functional roles of key cytokines and chemokines and their associated

receptors in the development of the main clinical outcomes associated with the cytokine storm. In addition to lung infections, the cytokine storm is a consequence of severe infections in the gastrointestinal tract, urinary tract, central nervous system, skin, joint spaces, and other sites.<sup>7</sup>

The commencement of a cytokine storm is followed by systemic production of IL-10, which is a marker of a counter-anti-inflammatory response that has been termed "immunoparalysis". It is associated with downregulation of neutrophil and monocyte function in the systemic circulation.<sup>5</sup> Although downregulation of systemic inflammation may prove to be beneficial in controlling systemic responses to local infections however, it has been observed that patients who survive the initial cytokine storm but subsequently die, are those who do not recover from immunoparalysis. Persistent downregulation of HLA-DR (a marker of immunosuppression) on monocytes 3-4 days after the onset of severe sepsis and cytokine storm, leads to high mortality rate, indicating a rationale for therapy to reverse immunosuppression under such circumstances.<sup>31,32</sup>

**Figure 3: Functional roles of key cytokines and chemokines and, their associated receptors in the development of main clinical outcomes associated with the cytokine storm**



TNF: Tumor necrosis factor; TNFR: Tumor necrosis factor receptor; IFN: Interferon; IFNAR: Interferon alpha receptor; IFNGR: Interferon gamma receptor; IL: Interleukin; IL1R: Interleukin receptor; ECM: Extracellular matrix

**Source:** Jennifer R. Tisoncik JR, Korth MJ, Cameron P. Simmons CP, Farrar J, Martin TR, Katze MG. Into the Eye of the Cytokine Storm. *Microbiol Mol Biol Rev*. 2012;76(1):16-32.



## Targeting the cytokine storm

Several acute infections may stimulate powerful and potentially destructive immune response. Therapeutic strategies targeting this response in order to reduce the self-inflicted damage initiated by the host in response to infection may prove to be beneficial. During a severe acute infection, modulation of the elements of immune response is required. There may be a need to enhance certain elements at times and suppress at other times.

### Implications for therapeutic strategies

Immunomodulatory drugs may decrease inflammation during infection with drug treatment and thus show therapeutic benefit. Sphingosine receptors play a vital role in innate immune responses, and sphingosine analogs have demonstrated potential for controlling the

cytokine storm caused by influenza virus.<sup>33,34</sup> Sphingosine-1-phosphate (S1P) receptor 1 suppresses immune cell recruitment through downregulation of cytokine and chemokine production by respiratory endothelial cells in the presence of S1P<sub>1</sub>-selective agonists, CYM-5442 and RP-002, including production of IFN-alpha, CCL2, IL-6, TNF-alpha, and IFN-gamma. An experimental study<sup>35</sup> showed that blunted innate chemokine and cytokine responses mediated by S1P<sub>1</sub>-selective agonists protected experimental models from lethal infection with pandemic H1N1 influenza virus.

Peroxisome proliferator-activated receptors (PPARs) are lipid-activated transcription factors. They are important regulators of lipid metabolism and inflammation. PPAR-gamma agonists downregulate the inflammatory response to virus-induced lung inflammation.<sup>36</sup> The findings of a study showed that treatment with

**Table 2: Evidence based observations of anti-inflammatory properties of immunomodulatory drugs**

| Type of therapeutic            | Drugs   | Immunomodulatory effects  |
|--------------------------------|---|---|
| COX inhibitor                  | Mesalamine, celecoxib   | Coadministration of COX inhibitors with zanamivir was comparatively more effective than antiviral monotherapy in reducing cellular infiltrate and improving survival of H5N1 virus-infected experimental models <sup>1,2</sup>  |
| CCR2 inhibitor                 | PF-04178903   | Increased survival of experimental models infected with influenza virus, and decreased lung immunopathology <sup>3,4</sup>  |
| Sphingosine receptor agonists  |   | Suppressed cytokine and chemokine production<br>Sphingosine receptors play a vital role in innate immune responses <sup>5</sup>   |
| Anti-TNF agents                |   | Mediator of pulmonary inflammation during influenza A viral pneumonia, reduced severity of pulmonary immunopathology and prolonged survival of A/PR/8-infected experimental models <sup>6</sup>   |
| PPAR-alpha/PPAR-gamma agonists | Gemfibrozil, pioglitazone, rosiglitazone, 15d-PGJ2, ciglitazone, troglitazone | 15d-PGJ2, ciglitazone, troglitazone decreased production of IL-alpha, IL-6 and TNF cytokines, CXCL8 and CCL5 chemokines and ICAM-1 in RSV-infected lung epithelial cells<br>Administration of gemfibrozil increased survival of experimental models infected with severe influenza <sup>7,8</sup> |

COX: Cyclo-oxygenase; TNF: Tumor necrosis factor; IL: Interleukin; PPAR: Peroxisome proliferator-activated receptor

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gemfibrozil targeted PPAR-alpha and thus reduced the inflammatory response. It improved the survival of experimental models infected with influenza H2N2 virus.<sup>37</sup> Combination therapy consisting of zanamivir, an inhibitor of the influenza virus neuraminidase, and celecoxib and mesalazine, inhibitors of inflammation, increased the survival of experimental models infected with a highly pathogenic strain of H5N1 influenza virus.<sup>38</sup> The cyclooxygenase-2 (COX-2) inhibitors, celecoxib and mesalazine also reduced the mortality rate of models infected with H5N1 influenza virus, when administered in combination with zanamivir.<sup>39</sup> These studies reveal that combination of antiviral and immunomodulatory drugs is more effective than antiviral monotherapy. The anti-inflammatory properties of various immunomodulatory drugs are mentioned in table 2.

Ulinastatin is a potent multivalent serine protease inhibitor. It is an effective therapeutic agent in the treatment of sepsis, and the most life-threatening

complications of critically ill patients. It has also been found to be effective in ameliorating sepsis-related acute lung injury, a syndrome most frequent and fatal in sepsis. The molecular mechanism investigation showed that ulinastatin's protection against ALI was probably related to the down-regulation of NF-kappaB activity and inhibition of TNF-alpha, IL-6 and elastase expressions in the lung tissue.<sup>40</sup>

## Conclusion

Cytokine storm develops as a consequence to any impairment in the production of cytokines. Several cytokines are associated with the cytokine storm, including interferons, interleukins, chemokines, CSFs and TNF. Balance between pro- and anti-inflammatory mechanisms is important in maintaining immune homeostasis. Absence or aberrant regulation of one or more of these regulatory mechanisms may contribute towards a cytokine storm. Over the last few decades,

### Advantages of ulinastatin in lung protection

Ulinastatin, also known as human urinary trypsin inhibitor, plays a key role in suppressing systemic inflammation and proteolytic process. Its potential benefits in acute pancreatitis and septic shock have been well established. Recent clinical trials have highlighted its advantages in lung protection, as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) share a common pathogenesis with sepsis, which is systemic inflammatory response syndrome.

The current study investigated the efficacy and safety of ulinastatin in patients with ALI and ARDS. A systematic review of randomized controlled trials of ulinastatin for ALI/ARDS was conducted. Twenty-nine randomized controlled trials with 1,726 participants were included in the study, with similar basic conditions. Oxygenation index, mortality rate [intensive care unit (ICU) mortality rate, 28-day mortality rate] and length of ICU stay were compared between ulinastatin group and conventional therapy group. Meta-analysis was performed by using Rev Man 5.1. Oxygenation index was reported in 26 studies (1552 patients). Ulinastatin had a significant effect in improving oxygenation [standard mean difference (SMD)=1.85, 95%CI: 1.42-2.29, P <0.00001, I<sup>2</sup>=92%]. ICU mortality and 28-day mortality were reported in 18 studies (987 patients) and 3 studies (196 patients), respectively.

The results showed:

- Ulinastatin significantly decreased the ICU mortality [I<sup>2</sup>=0%, RR=0.48, 95%CI: 0.38-0.59, number needed to treat (NNT)=5.06, P <0.00001]
- The 28-day mortality was not significantly affected (I<sup>2</sup>=0%, RR=0.78, 95%CI: 0.51-1.19, NNT=12.66, P=0.24)
- The length of ICU stay (6 studies, 364 patients) in the ulinastatin group was significantly lower than that in the control group (SMD=-0.97, 95%CI: -1.20--0.75, P <0.00001, I<sup>2</sup>=86%).

The efficacy of ulinastatin in ALI and ARDS was well demonstrated, though no information on safety was obtained.

**Source:** Yu-Xin Leng, Shu-Guang Yang, Ya-Han Song, Xi Zhu, Gai-Qi Yao. Ulinastatin for acute lung injury and acute respiratory distress syndrome: A systematic review and meta-analysis. *World J Crit Care Med.* 2014;3(1):34-41.



## Ulinastatin ameliorates cell injury of lung tissues in systemic hyperthermia

Hyperthermia is associated with systemic inflammatory response syndrome and aggravates injuries to various organs. It causes release of large amounts of inflammatory mediators and induces a systemic inflammatory response and immune dysfunction. The investigators of this study assessed the effects of ulinastatin administered at different time points on the cellular morphologies of the lung tissues of experimental models with systemic hyperthermia.

A total of 40 experimental models were randomly divided into five groups: The normal control group (C group), the hyperthermia group without medication (H group), the hyperthermia and ulinastatin pre-treatment group (HU group), the group treated with ulinastatin at 1 hour after hyperthermia (HU1 group), and the group treated with ulinastatin at 2 hours after hyperthermia (HU2 group). The systemic hyperthermia experimental model was established in a heating chamber with a biological oxygen supply. For the HU, HU1 and HU2 groups, ulinastatin ( $5 \times 10^4$  U/kg) was administered at different time points. For the C and H groups, an equivalent volume of normal saline was administered. During heating, the respiratory frequency and rectal temperature were measured and recorded once every 30 minutes. After 2.5 hours of heating, the wet/dry weight (W/D) ratio of the lung tissues was measured. Additionally, the cellular morphologies of the lung tissues were observed under light and electron microscopes.

The respiratory frequencies and lung tissue W/D ratios in the various hyperthermia groups were significantly higher than those in the C group. The respiratory frequencies and lung tissue W/D values of the HU and HU1 groups were significantly lower than those of the H group. Under the light microscope, the bronchial surrounding tissues of the HU and HU1 groups were loose, and the majority of the pulmonary alveolar structures were normal; the H and HU2 groups presented a number of changes, including pulmonary interstitial hyperemia, alveolar epithelial swelling and emphysema. Under the electron microscope, it was observed in the type II epithelial cells of the pulmonary alveoli of the H group that the mitochondria were swollen, the cell ridges were shortened, the microvilli were thin and increased, and the alveolar wall was thickened. Also, an increased number of infiltrating neutrophils were visible. In addition, the type II epithelial cells of the HU2 group also presented these changes to different extents and the changes in the HU and HU1 groups were the mildest.

These results revealed that early treatment with ulinastatin helps in decreasing edema and extent of cell injury of the lung tissue in systemic hyperthermia.

**Source:** Qin ZS, Tian P, Wu X, Yu HM, Guo N. Effects of ulinastatin administered at different time points on the pathological morphologies of the lung tissues of rats with hyperthermia. *Exp Ther Med*. 2014;7(6):1625-1630.

several trials have been conducted to reveal the cytokine dynamics during infections. Efficient therapeutic strategies including antiviral and immunomodulatory drugs show potential benefits in ameliorating inflammation. In addition, ulinastatin has emerged as an effective agent for sepsis treatment due to its anti-inflammation and anti-protease activities that ameliorate systemic disorders, prevent organ injuries and thus improve the survival outcomes of sepsis. Moreover, it also treats acute lung injury and acute respiratory distress syndrome effectively.

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# Viral Encephalitis: A review of causes, differential diagnosis and management

## Introduction

Encephalitis is an inflammatory condition affecting the brain. It is usually diffuse in nature. Virus is considered the most common and important etiological agent of encephalitis.<sup>1</sup> The time course of the viral encephalitis can be acute, subacute, or chronic. Several viruses may cause central nervous system (CNS) diseases with a broad range of clinical manifestations. It produces inflammation of the brain tissue, which may evolve to destruction of nerve cells, cause bleeding and brain damage, ultimately leading to severe encephalitis, such as hemorrhagic or necrotizing encephalitis, with a worse prognosis, resulting in serious sequelae or death.<sup>2</sup> Viruses responsible for encephalitis can be animal-borne, vector-borne or transmit from human-to-human; immunocompetent or immunosuppressed patients being the prime targets.<sup>3</sup> Alteration of normal immune function due to a previous viral infection may also result in encephalitis.<sup>1</sup> Among various types, encephalitis caused by herpes is the most prevalent infectious encephalitis, and the only one with a validated specific treatment.<sup>3</sup> The association of viral encephalitis with significant mortality and morbidity warrants early and appropriate diagnosis followed by an effective management approach.<sup>4</sup>

## Causes of viral encephalitis

Acute viral encephalitis is caused by a wide range of viruses. It may occur either in sporadic episodes or in outbreaks. Viral etiologic agents that have been identified to cause encephalitis include herpes virus, enterovirus, alpha virus, influenza A virus, rabies virus, HIV, flavi virus,

and Chandipura virus (figure 1).<sup>1,5,6</sup> Although precise data on the incidence of encephalitis caused by these viruses is lacking however, estimates for some of them have been well documented. Accumulating evidence suggests that herpes simplex virus (HSV) encephalitis is highly prevalent. It affects approximately 2,000 individuals annually in the USA.<sup>7,8</sup> Investigators have reported that HSV-1 is responsible for around 90% of cases of HSV encephalitis while remaining 10% are attributed to HSV-2 (figure 2). Encephalitis caused by HSV-2 is more common in immunocompromized individuals and neonates, in whom it causes a disseminated infection. It is noteworthy that molecular analyses of paired oral/labial and brain sites have revealed that HSV encephalitis can be the result of a primary infection, a reactivation of latent HSV, or a re-infection by a second HSV.<sup>7</sup> The findings of a polymerase chain reaction (PCR) based study demonstrated that upto a fifth of patients with HSV encephalitis may have mild or atypical disease caused by either HSV-1 or HSV-2, occurring particularly in immunocompromized individuals such as those with HIV infection.<sup>9</sup> Another study<sup>10</sup> in Finland also used PCR to detect various viruses in the cerebrospinal fluid (CSF) of over 3,000 patients who had infections of the central nervous system including encephalitis, meningitis, and myelitis. The results were startling, as varicella zoster virus (VZV), the cause of chickenpox and herpes zoster, was the most frequently detected virus (29%), followed by HSV and enteroviruses accounting for 11% of cases each, and influenza A virus observed in 7% of cases (figure 3).

Furthermore, the frequency and distribution of these viruses also depends on the geographical region.

## Deep sequencing: A rapid and accurate technique to identify causes of viral encephalitis

Fast and specific identification of pathogens enables early and effective treatment, especially in case of emerging infectious diseases and viral epidemics. This process is generally tedious and time-consuming thus making it ineffective in conventional settings. Although current diagnostic methods used in cases of infectious encephalitis successfully identify a specific microbiologic cause of the disease however, majority of cases actually have an infectious etiology but are misdiagnosed. Deep sequencing has emerged as a rapid and accurate technique to assist in the diagnosis of a wide variety of infectious diseases.

Deep sequencing is an effective and quick approach in the characterization of microbial DNA and RNA sequences in several samples. Investigators of the present study investigated frozen brain samples from seven deceased subjects with recent encephalitis, and fourteen normal brain served as control, to evaluate the presence of viral sequence. RNA from each sample was extracted, randomly reverse transcribed and sequenced. The sequence analysis was performed in a blinded fashion and confirmed with pathogen-specific PCR.

This analysis successfully identified measles virus sequences in two brain samples and herpes simplex virus type-1 sequences in three brain samples. No pathogen was identified in the other two brain specimens. These results were concordant with pathogen-specific PCR and partially concordant with prior neuropathological examinations, demonstrating that deep sequencing can accurately identify viral infections in frozen brain tissue.

**Source:** Chan BK, Wilson T, Fischer KF, Kriesel JD. Deep sequencing to identify the causes of viral encephalitis. *PLoS One*. 2014;9(4):e93993.

Disparity has been observed between Europe, Asia, and the USA. St Louis virus encephalitis, caused by a mosquito borne arbovirus, is prevalent in the midwestern and eastern states of the USA, and not in the UK. Japanese encephalitis is a major problem in Asia, and is the most important cause of epidemic encephalitis worldwide, accounting for around 15,000 deaths annually.<sup>11</sup> West Nile virus encephalitis and Nipah virus encephalitis have also emerged as the viral infections of the nervous system. The former is caused by the transport of neurovirulent strains of West Nile virus into the Western hemisphere, while the latter has resulted from the penetration of a newly recognized paramyxovirus across a species barrier from bat to pig, and represents the first large scale epizootic encephalitis involving direct animal to human transmission.<sup>8</sup>

### Differential diagnosis

Viral encephalitis is characterized by fever, headache, and clouding of consciousness along with seizures and focal neurology in some cases. However, differentiation between an infective viral encephalitis and a metabolic encephalopathy or acute disseminated encephalomyelitis remains a complicated issue. Therefore, diagnosis of infective viral encephalitis should be followed by

an investigative strategy in order to determine the likely cause.<sup>1</sup> Myriad of medical conditions result in encephalopathic illness that may simulate viral encephalitis, some of these are mentioned in box 1.<sup>6</sup>

### Distinguishing viral encephalitis from non-infective encephalopathy

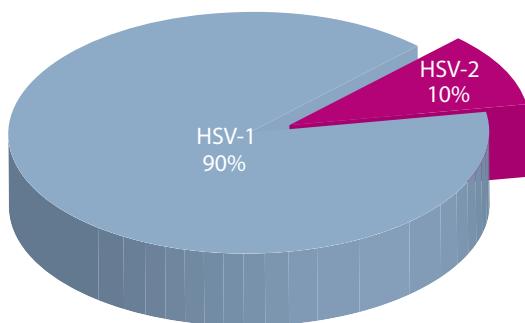
Differentiation of viral encephalitis from a non-infective encephalopathy is based on careful examination followed by cranial imaging to exclude a structural lesion. In addition, CSF examination may also be beneficial in distinguishing between the two conditions.<sup>12</sup> Presence or absence of focal signs and seizures, electroencephalographic (EEG) changes, normal or otherwise cranial imaging, and CSF profile, particularly, aid in making the distinction between these two conditions.<sup>1</sup> From a neuropathological perspective, inflammation is considered as an important factor to distinguish between viral encephalitis and non-infective encephalopathy. Patients with encephalopathy have focal or generalized dysfunction of neurons and supporting cells without inflammation, thus fever or headache is less likely to occur in these patients. Moreover, the inflammatory response engendered by infection results in both peripheral leukocytosis and CSF pleocytosis, which are

**Figure 1: Causes of viral encephalitis**

- Herpes simplex virus (HSV-1, HSV-2)
- Other herpes viruses: Varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpes virus 6 (HHV6)
- Adenoviruses
- Influenza A
- Enteroviruses, poliovirus
- Measles, mumps and rubella viruses
- Rabies
- Arboviruses (for example: Japanese B encephalitis, St Louis encephalitis virus, West Nile encephalitis virus, Eastern, Western, and Venezuelan equine encephalitis virus, Tick borne encephalitis viruses)
- Bunyaviruses (for example: La Crosse strain of California virus)
- Reoviruses (for example: Colorado tick fever virus)
- Arenaviruses (for example: Lymphocytic choriomeningitis virus)

**Source:** Coyle PK. Postinfectious encephalomyelitis. In: Davis LE, Kennedy PGE, eds. Infectious diseases of the nervous system, 1st ed. Butterworth-Heinemann, 2000:83-108.

characteristic features of viral encephalitis. Direct viral infection often results in areas of focal CNS injury that are reflected in focal abnormalities on magnetic resonance imaging (MRI) or other neuroimaging studies and in focal discharges on EEG. Counterintuitively, the more diffuse nature of brain dysfunction in encephalopathy typically induces generalized slowing without focal features on EEG and nonfocal neuroimaging studies. Although encephalopathy usually results from noninfectious processes however, some infectious agents may induce CNS dysfunction in the absence of either direct invasion or induction of a postinfectious immune-mediated

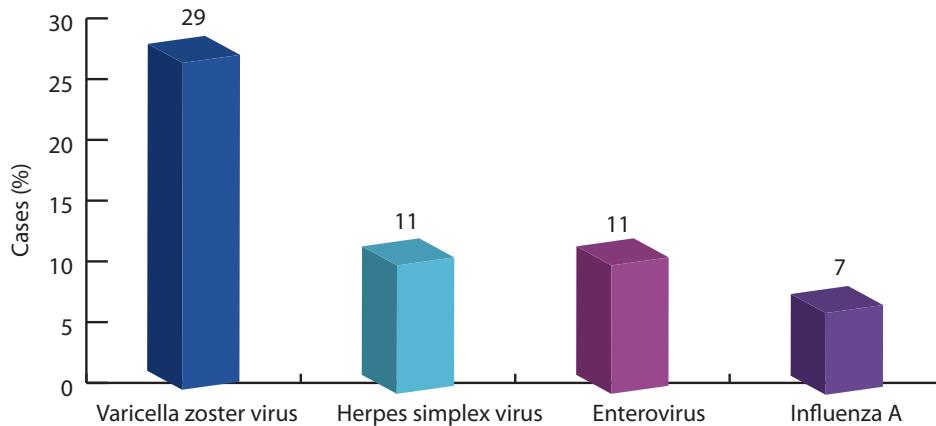
**Figure 2: Herpes simplex virus (HSV) encephalitis cases occurring due to HSV-1 and HSV-2**

**Source:** Whitley R, Lakeman AD, Nahmias A, Roizman B. DNA restriction-enzyme analysis of herpes simplex virus isolates obtained from patients with encephalitis. *N Engl J Med*. 1982;307(17):1060-2.

response. Viral culture and/or PCR is unlikely to be positive in cases of encephalopathy.<sup>13</sup> Common distinguishing features between viral encephalitis and non-infective encephalopathy are mentioned in table 1.<sup>12</sup>

### Distinguishing viral encephalitis from acute disseminated encephalomyelitis

Acute disseminated encephalomyelitis is also known as post-infectious encephalomyelitis. It may be attributed to a systemic infection affecting the respiratory or gastrointestinal systems, an infection that may be a childhood exanthema such as measles, rubella or chickenpox, or following a vaccination within the preceding four weeks.<sup>12</sup> A strong body of evidence suggests an immune pathogenesis of this disorder, with an abnormal immune reaction directed against normal brain. Potential immune mechanisms include molecular mimicry between epitopes expressed by myelin antigens and viral or other pathogens, immune dysregulation, superantigen induction, and a direct result of the infection.<sup>6</sup> It is noteworthy that this disorder can also present with a very restricted neurological picture such as transverse myelitis, optic neuritis, and cerebellar ataxia.<sup>14</sup> Immediate diagnosis of acute disseminated encephalomyelitis may not be always possible however, recording a meticulous history followed by careful examination and investigations may assist in distinguishing acute disseminated encephalomyelitis from infectious encephalitis. These differences have been summarized in table 2.<sup>12</sup>

**Figure 3: Viruses in the cerebrospinal fluid of patients having encephalitis, meningitis and myelitis**

**Source:** Koskineni M, Rantalaaho T, Piiparin H, von Bonsdorff CH, Färkkilä M, Järvinen A, Kinnunen E, Koskineni S, Mannonen L, Muttineni M, Linnavauri K, Porras J, Puolakkainen M, Räihä K, Salonen EM, Ukkonen P, Vaheri A, Valtonen V; Study Group. Infections of the central nervous system of suspected viral origin: a collaborative study from Finland. *J Neurovirol.* 2001;7(5):400-8.

#### Box 1: Common causes of encephalopathy

- Anoxic/ischemic
- Metabolic
- Nutritional deficiency
- Toxic
- Systemic infections
- Critical illness
- Malignant hypertension
- Mitochondrial cytopathy (Reye's and MELAS syndromes)
- Hashimoto's encephalopathy
- Paraneoplastic
- Neuroleptic malignant syndrome
- Traumatic brain injury
- Epileptic (non-convulsive status)

**Source:** Coyle PK. Postinfectious encephalomyelitis. In: Davis LE, Kennedy PGE, eds. Infectious diseases of the nervous system, 1st ed. Butterworth-Heinemann, 2000:83-108.

Aciclovir has emerged as an efficacious treatment option in HSV encephalitis. Accumulating evidence have revealed the efficacy of aciclovir in treating patients with HSV encephalitis. It reduced the six month mortality to about 20%, one quarter to one third of patients required long term supportive care and about 50% returned to normal life.<sup>15</sup>

Aciclovir is an analogue of 2'-deoxyguanosine and selectively inhibits viral replication. It metabolizes to aciclovir triphosphate to exert its antiviral effect. Initially, monophosphorylation of aciclovir occurs and is catalyzed by a viral thymidine kinase induced in cells selectively infected by HSV, VZV or by a phosphotransferase produced by cytomegalovirus.<sup>16</sup> Host enzymes subsequently phosphorylate the monophosphate to diphosphate and triphosphate. Aciclovir triphosphate inhibits the synthesis of viral DNA by competing with a 2'-deoxyguanosine triphosphate as a substrate for viral DNA polymerase. Viral DNA synthesis is arrested once aciclovir (rather than 2'-deoxyguanosine) is inserted into the replicating DNA. The incorporation of aciclovir into viral DNA is an irreversible process and it also inactivates viral DNA polymerase (figure 4). The potency of aciclovir triphosphate to inhibit HSV-1 DNA polymerase is 30-50 times greater than its ability to inhibit human cellular alpha-DNA polymerase.<sup>17</sup>

Aciclovir has a relatively short half life in plasma and more than 80% of aciclovir in circulation is excreted

## Management

Timely initiation of treatment is crucial while relevant molecular and serological tests are being performed. Wherever possible, specific treatment must be targeted to the suspected or identified etiological agent. Over the last few decades, lack of an effective antiviral treatment has raised the mortality rate of HSV encephalitis to 70%, and most survivors have suffered from severe neurological deficits. However, antiviral therapy with

**Table 1: Common differences between encephalopathy and encephalitis**

|                            | Encephalopathy        | Encephalitis                            |
|----------------------------|-----------------------|---|
| <b>Clinical features</b>   |                       |   |
| Fever                      | Uncommon              | Common                                  |
| Headache                   | Uncommon              | Common                                  |
| Depressed mental status    | Steady deterioration  | May fluctuate                           |
| Focal neurologic signs     | Uncommon              | Common                                  |
| Types of seizures          | Generalized           | Generalized or focal                    |
| <b>Laboratory findings</b> |                       |   |
| Blood                      | Leukocytosis uncommon | Leukocytosis common                     |
| CSF                        | Pleocytosis uncommon  | Pleocytosis common                      |
| EEG                        | Diffuse slowing       | Diffuse slowing and focal abnormalities |
| MRI                        | Often normal          | Focal abnormalities                     |

CSF: Cerebrospinal fluid; EEG: Electroencephalogram; MRI: Magnetic resonance imaging

**Source:** Davis LE. Diagnosis and treatment of acute encephalitis. *The Neurologist*. 2000;6:145-59.

unchanged in urine, thus renal impairment can rapidly precipitate aciclovir toxicity.<sup>18</sup> Studies have consistently confirmed that aciclovir is most effective when given early in the clinical course of HSV encephalitis before the patient becomes comatose and reduces both mortality and morbidity in treated patients.<sup>14,19</sup> The standard dose of aciclovir for HSV encephalitis is 10 mg/kg three times daily (30 mg/kg/day) for 14 days. The dose for neonatal HSV encephalitis is 60 mg/kg/day. The duration of treatment is 21 days for immunosuppressed patients

(box 2). Aciclovir is effective against encephalitis due to HSV-1, HSV-2, and VZV.<sup>16</sup> Doses of aciclovir in VZV encephalitis are similar to HSV encephalitis.

Despite its remarkable safety, treatment of HSV encephalitis with aciclovir has two potential drawbacks. Firstly, prescribing aciclovir may delay or obscure the actual diagnosis (if not HSV encephalitis) due to a false sense of security. Thus, diagnosis of other infective encephalitis, acute disseminated encephalomyelitis, or non-infective encephalopathies like reye's syndrome,

**Table 2: Comparison of acute disseminated encephalomyelitis and infectious encephalitis**

|                                | Acute disseminated encephalomyelitis  | Infectious encephalitis  |
|--------------------------------|---|--|
| <b>Clinical features</b>       |   |  |
| Most common age                | Children  | Any age  |
| Recent vaccination             | Common  | Uncommon   |
| Prodromal illness              | Usually   | Occasionally   |
| Fever                          | May occur   | Common   |
| Visual loss (one or both eyes) | May occur   | Uncommon   |
| Spinal cord signs              | May occur   | Rare   |
| <b>Laboratory findings</b>     |   |  |
| Blood                          | Leukocytosis occasionally occurs  | Leukocytosis common  |
| MRI (T2 weighted)              | Multiple focal areas of hypertensity that are the same and may involve white matter of both hemispheres, basal ganglia, brainstem, cerebellum, and spinal cord. Lymphocytic pleocytosis, elevated protein, normal glucose, and negative cultures. Red blood cells seen in acute hemorrhagic leukoencephalitis | One or more diffuse areas of hypertensity involves the grey matter of both cerebral cortices and its underlying white matter and, to a lesser extent, basal ganglia, brainstem, and cerebellum. Lymphocytic pleocytosis, elevated protein, normal glucose, and negative cultures. Red blood cells may be seen in herpes simplex encephalitis |

**Source:** Davis LE. Diagnosis and treatment of acute encephalitis. *The Neurologist*. 2000;6:145-59.



hashimoto's encephalopathy may be delayed or even missed. Secondly, aciclovir is not completely safe and can precipitate a toxic encephalopathy that can confound the diagnosis of acute encephalitis if this has not been made before the treatment was initiated.<sup>16</sup>

Furthermore, there is a dearth of studies on the clinical response of cytomegalovirus encephalitis to antiviral drugs. The efficacy of aciclovir is not well established in cytomegalovirus encephalitis. However, combination therapy with ganciclovir (5 mg/kg intravenously twice daily) with or without foscarnet (60 mg/kg every eight hours or 90 mg/kg every 12 hours) may prove to be beneficial, cidofovir is a possible alternative. Antiretroviral therapy must be added or continued in HIV infected patients.<sup>14</sup>

### Supportive therapy

Supportive therapy for acute encephalitis is the mainstay of treatment strategy. Seizures can be controlled with intravenous fosphenytoin. Raised intracranial pressure must be appropriately treated, following principles for the medical management. Careful attention must be paid to the maintenance of respiration, cardiac rhythm, fluid

### Box 2: Aciclovir treatment in HSV encephalitis

- Specific and highly effective
- Safe, but requires dose adjustment for renal function
- Treatment increase survival likelihood to 65-100%, if disease is present for four days or less
- Dose is 10 mg/kg every eight hours for at least 14 days in an immunocompetent host

#### Sources:

1. Balfour HH. Antiviral drugs. *N Engl J Med.* 1999;340:1255-68.
2. Whitley RJ, Gnann JW. Viral encephalitis: familiar infections and emerging pathogens. *Lancet.* 2002;359:507-14.

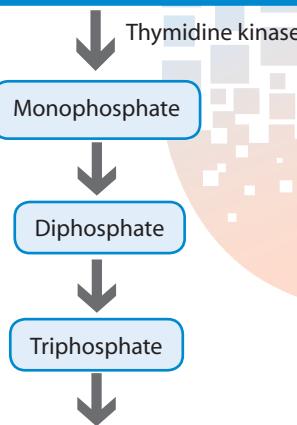
balance, prevention of deep vein thrombosis, aspiration pneumonia, and secondary bacterial infections. Since toxicity with antiviral drugs is frequently encountered in clinical practice, thus appropriate blood counts and biochemical parameters must be closely monitored. Each dose of aciclovir should be given intravenously slowly as an infusion over at least one hour and the dose may require adjustment based on renal function. All cases of acute encephalitis must be hospitalized and should have access to intensive care unit equipped with mechanical ventilators. Isolation for patients with community acquired acute infective encephalitis is not required; rabies encephalitis, however, is an exception. Consideration of isolation should also be given for severely immunosuppressed patients, patients with exanthematous encephalitis, and those with a potentially contagious viral hemorrhagic fever. Delay in treatment commencement, particularly beyond 48 hours after hospital admission, are associated with a worse prognosis.<sup>14</sup>

### Conclusion

Acute infective encephalitis is usually caused by virus. Herpes simplex encephalitis is one of the most common sporadic acute viral encephalitis. The early diagnosis and correct management of viral encephalitis can reduce mortality and neurological sequelae. Appropriate investigations and supportive care form the integral part of the management strategy. The availability of aciclovir, an excellent anti-HSV therapy, has led to early initiation of the treatment with substantial improvement in the clinical outcome of HSV encephalitis. However, even with recent treatment advances, potentially devastating outcomes are still possible.

**Figure 4: Mechanism of action of aciclovir**

ACICLOVIR (analogue of 2'-deoxyguanosine)



Inhibits synthesis of viral DNA (irreversible process) and inactivates viral DNA polymerase

**Source:** Balfour HH Jr. Antiviral drugs. *N Engl J Med.* 1999; 340:1255-68.



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# A review of malaria and significant role of ulinastatin in its management

## Introduction

Malaria, accounts for a major cause of morbidity and mortality worldwide. It is one of the most deadly diseases. Its foremost impact is seen in tropical and subtropical regions of Africa, Central and South America, Asia and Oceania. A massive 8,81,000 deaths every year are attributed to malaria which majorly involves children, with 9 out of 10 deaths occurring in Sub Saharan Africa.<sup>1</sup> Malaria is caused by four parasites, *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax*, and *Plasmodium malariae*. *P. falciparum* causes the most serious and fatal form of malaria. The parasite is transmitted by the bite of female *anopheles* mosquito and also by blood transfusions and transplacentally. A tremendous geographic variation in intensity of transmission and risk of infection is associated with this disease. The disease is preventable and treatable; a good understanding of the disease transmission, vector, clinical presentation of the disease and population at risk can help alleviate many cases and also help in strategic control of the disease. Some key points related to malaria are summarized in box 1.<sup>1-3</sup>

## The intolerable burden of malaria

The global disease burden of malaria is colossal. According to the disability-adjusted life years (DALYs), an aggregate measure of premature mortality, morbidity, and disability, nearly five billion episodes of clinical malaria illness occur throughout the world, Africa bearing 90% of this disease burden.<sup>4</sup> According to WHO report 2012, approximately 3.3 billion people were at risk of developing malaria in the year 2011. Children under the age of 5 years and

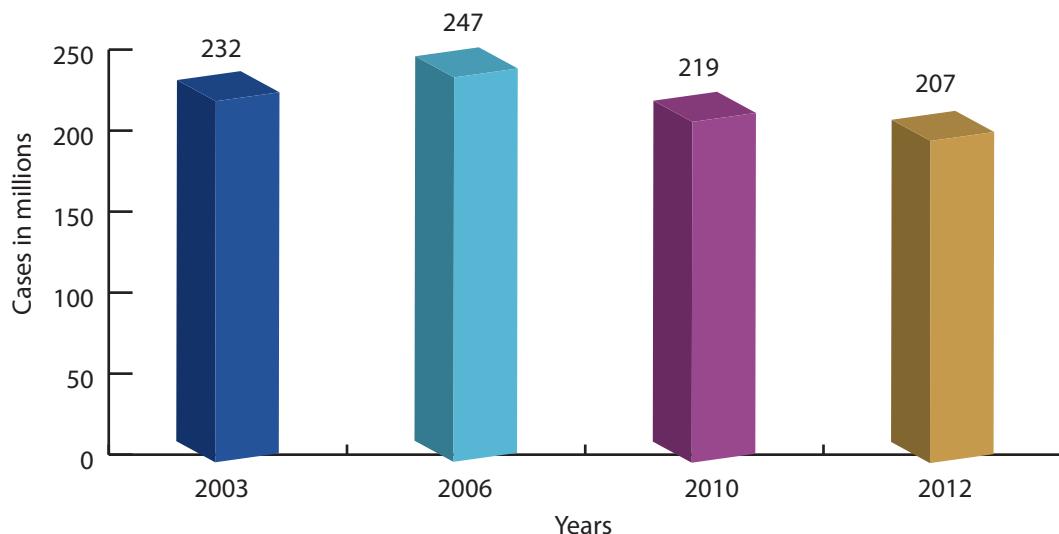
### Box 1: Malaria key points

- A large number of cases are seen in tropical and sub tropical regions of Africa, Central and South America and Asia
- Malaria is caused by four different parasite; *P. falciparum*, *P. ovale*, *P. vivax*, *P. malariae*
- Malaria is transmitted by the bite of female *anopheles* mosquito, blood transfusions and transplacentally
- Risk of infection and intensity of transmission varies according to geographical area
- Pregnant women and very young children are highly vulnerable to malaria
- It is difficult to distinguish malaria clinically from other febrile illnesses due to nonspecific symptoms
- Repeated exposure and infection of malaria can produce partial immunity.

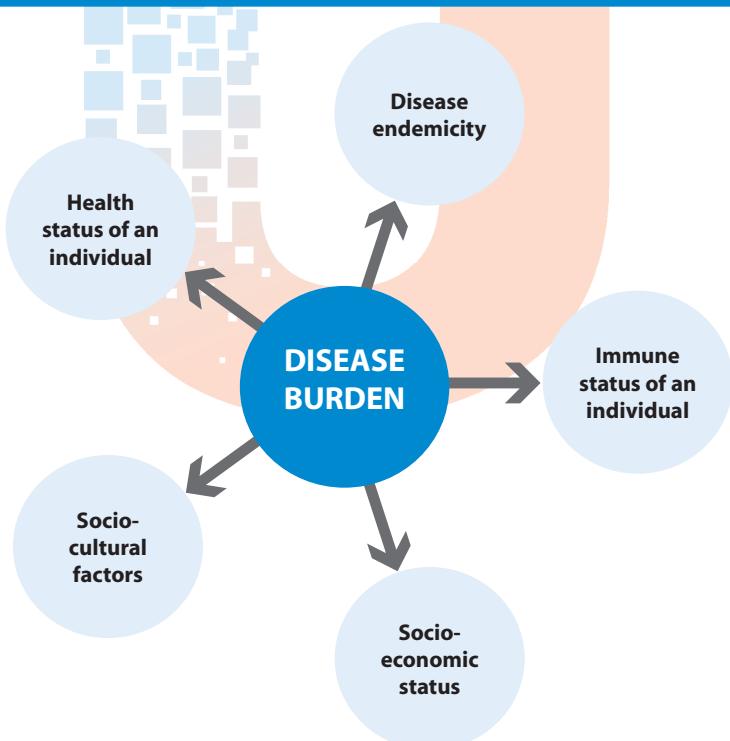
**Source:** Bloland PB, Williams HA; National Research Council (US) Committee on Population; Program on Forced Migration and Health at the Mailman School of Public Health, Columbia University. Malaria Control during Mass Population Movements and Natural Disasters. Washington (DC): National Academies Press; 2002. 3, Epidemiology of Malaria. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK221152/>. Accessed on: 2.8.2014.

pregnant women are severely affected by malaria.<sup>5</sup> Malaria cases and deaths reported in year 2003 where 232 million which increased to 247 million in 2006. This number was 219 millions in 2010 and 207 million in 2012 (figure1).<sup>1,5-7</sup>

The disease burden may inflict challenges in various realms like economic development, effects on productivity, relentless increase in morbidity and mortality and difficulty in meeting demand to combat disease. Various factors influencing disease burden in malaria<sup>8</sup> are depicted in

**Figure 1: Intolerable burden of malaria****Sources:**

1. Kokwaro G. Ongoing challenges in the management of malaria. *Malar J.* 2009;8 Suppl 1:S2.
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**Figure 2: Factors impacting disease burden in malaria**

**Source:** Jones CO, Williams HA. The social burden of malaria: what are we measuring? *Am J Trop Med Hyg.* 2004;71(2 Suppl):156-61.



figure 2. Certain risk factors predispose to cause increased risk for malaria which include unplanned mass movement of people, poor or no housing, overcrowding, proximity to livestock, malnutrition, immune status of an individual, socio-economic status and availability and accessibility to health care facilities.<sup>2</sup>

## Pathogenesis of malaria

Malaria is usually transmitted by the bite of female *Anopheles* mosquito. However, it may be transmitted during blood transfusions, nosocomial transmission or congenital transmission. In malaria transmitted by mosquito bite; the parasite *P. falciparum* is present in saliva of the mosquito. The infective sporozoites are passed into the human blood stream when mosquito bites. The sporozoites disappear from the blood stream and enter hepatocytes within 45 minutes of infection. In the hepatocytes, the sporozoites undergo asexual reproduction (exoerythrocytic schizogony) to form schizonts (collection of numerous merozoites). In 6-14 days schizonts rupture and release merozoites into the blood stream. This parasitic development phase in the hepatocytes is known as hepatic schizogony and lasts between 5.5 to 15 days depending on the parasite involved (*P. falciparum* or *P. malariae*). No clinical symptoms are seen in this phase of parasitic development. The merozoites released into the blood stream rapidly attack the red blood cells (RBCs) and start asexual multiplication (erythrocytic schizogony). These merozoites mature to form trophozoites and schizonts within the red blood cells. The RBCs then rupture; releasing merozoites into the blood stream which again invade new cells. This release marks the onset of clinical symptoms and the pathophysiological process of malaria. Thus, cycle continues till the infection is treated or immune response clears the infection or the individual dies. In this cycle parasites of *P. vivax* and *P. ovale* may remain dormant for few months to few years in the form of hypnozoites, and cause periodic relapses of peripheral parasitemia and illness. Additionally, gametocytes are also produced during the cycle which can be ingested by mosquito during the blood meal; undergo sexual reproduction in the mosquito and form sporozoites which then enter the salivary glands of mosquito, thus mosquitoes become infective to humans (figure 3).<sup>2,9</sup>

## Role of cytokines

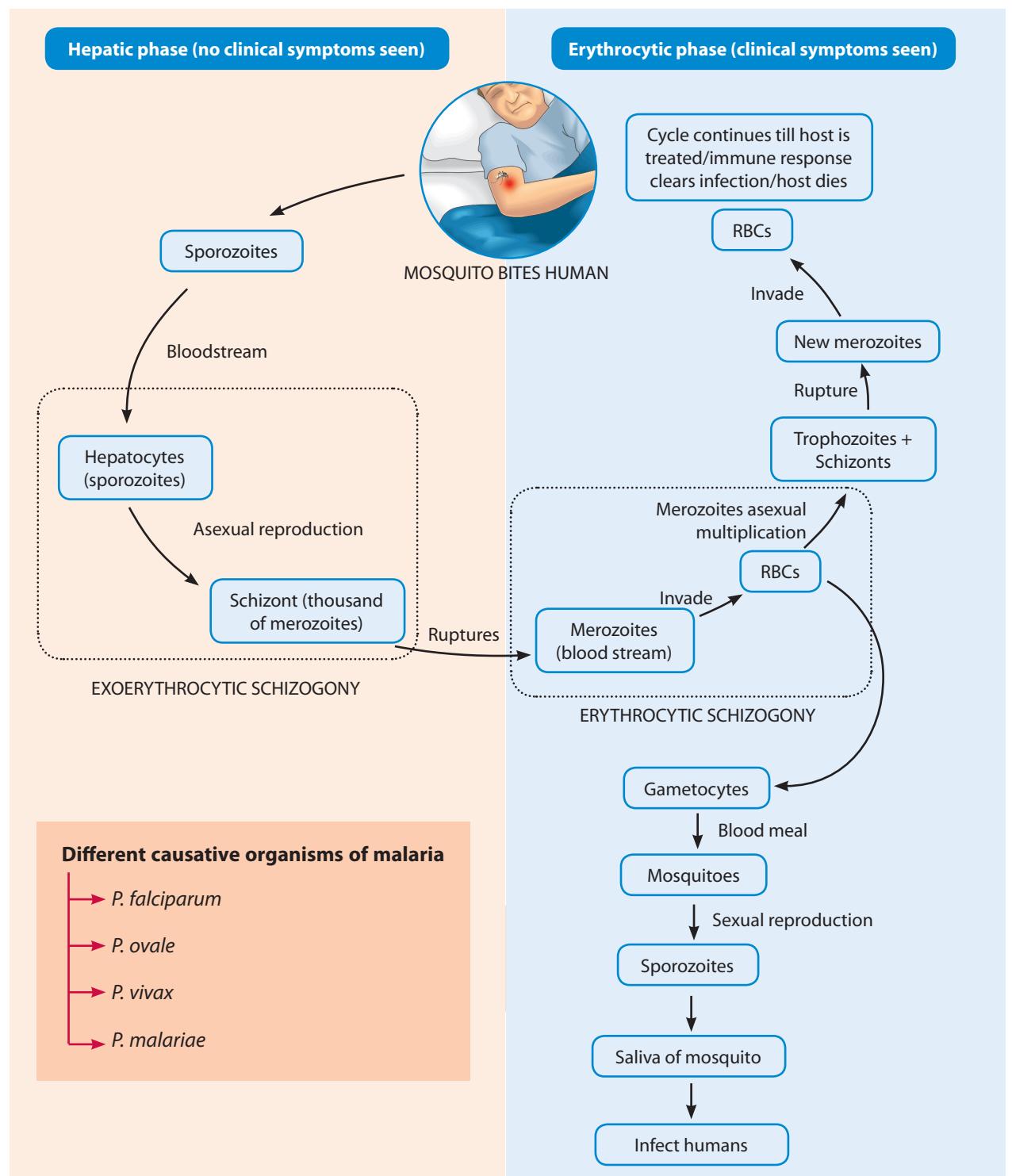
Many signs and symptoms seen during malaria may be attributed to the activation of cytokines cascade.<sup>9</sup> A delicate balance between inflammatory and regulatory

cytokines is required to prevent expression of pathologic symptoms in humans and also clear malaria parasite without any major consequences.<sup>10</sup> Innate and adaptive immunity both play a major role in malaria, these function to provide adequate protection from the infection. The paradigm is rather unclear but it is likely that early production of tumor necrosis factor (TNF)-alpha, interleukin (IL)-6, IL-12, interferon (IFN)-gamma and other inflammatory cytokines help in fast clearance of *P. falciparum*, mount resistance to the infection and protect from the clinical episodes. These appear to inhibit the growth of parasite and invigorate phagocytosis to enhance clearance of parasitized erythrocytes. And once the parasitemia is controlled IL-10 and transforming growth factors (TGF)-beta, the regulatory cytokines play a protective role by reducing the risk of severe disease. The chemotactic cytokines (chemokines) mediate the innate and adaptive immune response. They induce the chemotaxis of immune cells to the site of infection by inducing the migration of leukocytes that activate corresponding receptors on responsive cells. Plasma levels of TNF and nitric oxide (NO) along with carbon monoxide (CO) have an ambiguous role in malaria infection. They are associated with resolution of clinical symptoms and parasite clearance.<sup>10,11</sup> Inflammatory cytokines induce the production of inducible nitric oxide synthase (iNOS) and hemoxygenase-1 (HO-1) which mediate the production of NO and CO, respectively. Both, NO and CO play a vital role in maintaining inflammatory homeostasis, by inhibiting the generation of TNF. The inhibitory effect of CO is similar to that of IL-10; high levels of IL-10 are commonly associated with malaria and are proposed to suppress severity of the disease by inhibiting inflammatory effect of TNF. The sequence of IL-10 inducing HO-1 which generates CO that stimulates TNF is a plausible explanation of this effect. HO-1 is now regarded as an integral part of inflammatory chain besides its antioxidant effects. NO mediated homeostasis which inhibits growth of parasite, by down regulating TNF production via inducible nitric oxide synthase (iNOS), constitutes as the negative feedback mechanism in the cytokine cascade (figure 4). However, NO mediated induction of pathology in disease is also an unclear role of NO in this cascade.<sup>12</sup>

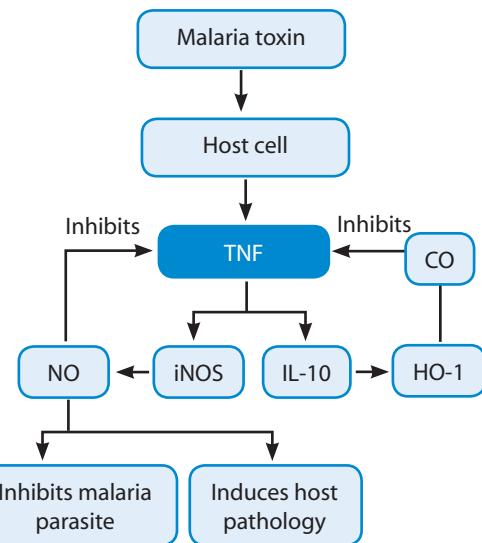
## Clinical manifestations of malaria

The clinical features are species specific and region specific. They depend on the infective dose and vary in individuals according to their immune status and severity of disease. The incubation period (interval between infection and the onset of symptoms) is 9-30 days or longer.

Figure 3: Pathogenesis of malaria

**Sources:**

1. Bloland PB, Williams HA; National Research Council (US) Committee on Population; Program on Forced Migration and Health at the Mailman School of Public Health, Columbia University. Malaria Control during Mass Population Movements and Natural Disasters. Washington (DC): National Academies Press; 2002. 3, Epidemiology of Malaria. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK221152/>. Accessed on: 2.8.2014.
2. Bartoloni A, Zammarchi L. Clinical aspects of uncomplicated and severe malaria. *Mediterr J Hematol Infect Dis*. 2012;4(1):e2012026.

**Figure 4: Role of cytokines in malaria**

iNOS: Inducible nitric oxide synthase; TNF: Tumor necrosis factor; IL-10: Interleukin-10; HO-1: Heme oxygenase-1; NO: Nitric oxide; CO: Carbon monoxide.

**Source:** Clark IA, Alleva LM, Mills AC, Cowden WB. Pathogenesis of malaria and clinically similar conditions. *Clin Microbiol Rev*. 2004;17(3):509-39.

Incubation period in malaria due to blood transfusion is shorter with symptoms developing within 10 days for *P. falciparum* and 14 days for *P. vivax*. Fever is the hallmark of malaria, including other prodromal symptoms (box 2) like malaise, anorexia, headache, backache, nausea and

#### Box 2: Common prodromal symptoms in malaria

- Fever
- Malaise
- Anorexia
- Lassitude
- Dizziness
- Headache
- Backache
- Myalgias
- Nausea
- Vomiting
- A chilling sensation

#### Sources:

1. Bartoloni A, Zammarchi L. Clinical aspects of uncomplicated and severe malaria. *Mediterr J Hematol Infect Dis*. 2012;4(1):e2012026.
2. Mohapatra MK, Patra P, Agrawala R. Manifestation and outcome of concurrent malaria and dengue infection. *J Vector Borne Dis*. 2012;49(4):262-5.

#### Box 3: Clinical features of severe malaria

- Generalized weakness
- Impaired consciousness
- Multiple convulsions (>2 episodes in 24 hours)
- Circulatory collapse or shock
- Difficulty in breathing, respiratory distress
- Evidence of vital organ dysfunction including clinical jaundice
- Abnormal spontaneous bleeding
- Pulmonary edema (radiological)

**Source:** Bartoloni A, Zammarchi L. Clinical aspects of uncomplicated and severe malaria. *Mediterr J Hematol Infect Dis*. 2012;4(1):e2012026.

vomiting.<sup>9, 13</sup> Fever in malaria is initially irregular and accompanied by shivering and mild chills. The typical malaria paroxysm occurs in three stages; cold stage, hot stage and sweating stage. The cold stage typically lasts for 10-30 minutes, the patient feels extreme cold and shivering, the skin appears cold, dry, pale and cyanosed and fever rises gradually to a peak. As the shivering subsides the hot stage starts. In the hot stage, the skin is hot and flushing of face occurs. The temperature may reach hyperpyrexial levels, accompanied by vomiting, headache, altered consciousness and extreme thirst. Within 2-6 hours, the terminal sweating stage begins which lasts for 2-3 hours and is characterized by profuse sweating, initially at the temples and later generalized. Subsequent to this the temperature falls rapidly and the patient feels well, yet tired. The entire paroxysm frequently begins in late afternoon or evening, and lasts 6-10 hours. Physical examination of the patient may reveal hepatomegaly and splenomegaly. Repeated exposure to the parasite may produce immunity in some subjects but severe form of malaria also exist which may present severe complications in non immune patients. Severe malaria involves the central nervous system (cerebral malaria), renal system (acute renal failure), pulmonary system (respiratory failure), and/or hematopoietic system (severe anemia).<sup>9</sup> Clinical features seen in severe malaria are summarized in box 3.

#### Diagnostic approach to malaria

Diagnostic approach to malaria encompasses clinical and laboratory diagnosis along with newer molecular diagnostic techniques. In routine outpatient settings clinical diagnosis is least expensive and the accuracy of diagnosis depends on variables like endemicity, age group or malaria season. No universal method of clinical diagnosis can be established owing to a range



#### Box 4: Features of microscopic diagnosis

- It can identify different species of malaria parasite
- It has good sensitivity and specificity
- It is easy to perform and requires operator expertise
- It is inexpensive
- It can detect 10-100 parasites/ microlitre of blood

#### Sources:

1. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). *Am J Trop Med Hyg.* 2007;77(6 Suppl):119-27.
2. Ryan ET. Malaria: epidemiology, pathogenesis, diagnosis, prevention, and treatment—an update. *Curr Clin Top Infect Dis.* 2001;21:83-113.

of non specific overlapping symptoms of the disease as discussed previously. Clinical diagnosis requires affirmative support of laboratory diagnosis to establish the disease and treatment. The laboratory diagnosis includes the gold standard microscopic technique of Giemsa-stained blood smears. Giemsa- stained peripheral blood smear when viewed under a light microscope may reveal different stages of parasite including merozoites, trophozoites with multiple infected red blood cells and schizonts.<sup>9</sup> Giemsa microscopy is regarded as the most acceptable diagnostic technique as it is inexpensive, can identify different species, and is sensitive and specific (box 4).<sup>14,15</sup> Besides microscopic diagnosis, routine blood examination may help in prompt diagnosis of malaria. Laboratory findings in severe malaria are summarized in box 5.<sup>16</sup>

Other laboratory diagnosis include rapid diagnostic technique (RDT) which detects malaria parasite even in a small amount of blood, by immunochromatographic assay with monoclonal antibodies targeting parasite antigen impregnated on a test strip. The results are quick, technique is simple to perform and easy to interpret.<sup>14</sup> Even though traditional malaria diagnostic techniques are useful yet they have many disadvantages to their share. Thus, new laboratory techniques having high sensitivity and specificity without subjective variations were urgently required. Molecular diagnostic techniques are a step towards meeting this demand. These include polymerase chain reaction (PCR), mass spectrometry (MS), flow cytometric (FCM) assay, microassay and loop-mediated isothermal amplification (LAMP) techniques. Characteristics of diagnostic techniques used in malaria detections are summarized in table 1.

#### Box 5: Laboratory findings in severe malaria

- Hypoglycemia (blood glucose < 40 mg/dl)
- Hemoglobinuria
- Severe normocytic anaemia (hemoglobin < 5 g/dl)
- Thrombocytopenia
- Metabolic acidosis (plasma bicarbonate < 15 mmol/l)
- Hyperparasitemia
- Hyperlactatemia (lactate > 5 mmol/l)
- Increased serum creatinine > 265 µmol/l
- Hyponatremia
- Increased C-reactive protein
- Increased procalcitonin levels

#### Sources:

1. Bartoloni A, Zammarchi L. Clinical aspects of uncomplicated and severe malaria. *Mediterr J Hematol Infect Dis.* 2012; 4(1):e2012026.
2. Koshy JM, Koshy J. Clinical profile of cerebral malaria at a secondary care hospital. *J Family Med Prim Care.* 2014;3(1):54-7.

## Management of malaria

There is a strong and urgent need to reduce the burden of malaria; it can help in effective control and treatment of malaria. The key is to combine preventive and therapeutic measures. Preventive measures that target vector need a good understanding of the vector biology and behavior. Methods like long-lasting insecticide-treated bed nets and indoor residual sprays control the vector.<sup>1</sup> Along with preventive measures, many chemotherapeutic agents are also available. These agents are required to be used judiciously owing to an emerging resistance of many of the agents in malaria endemic areas. Various therapeutics available includes chloroquine, mefloquine, doxycycline, artesunate, atovaquone, proguanil, primaquine and quinine. These agents may be used alone or in combination. The combination is preferred in areas where resistance to one or more agents has emerged. Few antimalarial drugs and their features are enlisted in table 2.<sup>2,15</sup> Chloroquine has been the drug of choice, for prophylaxis in individuals at high risk of developing malaria. Mefloquine is a drug of choice in USA for patients likely to develop chloroquine-resistant malaria. The efficacy of this drug exceeds 90-95% when used as a prophylactic agent. This drug is usually well tolerated, can be used in children and has fewer side-effects. Doxycycline is also a drug available for prophylaxis and treatment of malaria, although its efficacy is equivalent to mefloquine, but its use is not permitted in children below 8 years of age and pregnant females. Atovaquone and proguanil are used concomitantly as rapid resistance to atovaquone is observed when used

**Table 1: Characteristics of diagnostic techniques used in malaria detection**

| <b>Parameters →<br/>Diagnostic method used ↓</b> | <b>Principle of method</b>  | <b>Sensitivity and specificity</b>                            | <b>Time taken (minutes)</b>         | <b>Detection limit (parasites/ µl)</b> | <b>Expertise required</b> | <b>Cost of instrument</b> |
|--|---|---|-------------------------------------|--|---------------------------|---------------------------|
| <b>PBS</b>                                       | Visualization of parasite in stained blood smear under a light microscope   | Depends on good technique, reagent and skills of the observer | 30-60                               | Routinely > 50                         | High in non endemic areas | Low                       |
| <b>RDTs</b>                                      | Detection of parasite antigen or enzyme                                     | Moderate if more than 100 parasite/ µl                        | 10-15                               | 50-100                                 | Low                       | Moderate                  |
| <b>PCR</b>                                       | Specific amplification of malaria DNA                                       | Excellent   | 45-360 depending on the method used | 1-5                                    | High                      | Expensive                 |
| <b>LAMP</b>                                      | Detection of turbidity by a turbidity meter after amplifying DNA sequences  | Excellent   | <60                                 | >5                                     | High                      | Expensive                 |
| <b>FCM</b>                                       | Detection of hemozoin by flow cytometer                                     | Variable sensitivity, high specificity                        | Automated                           | Poor correlation with parasitemia      | High                      | Expensive                 |
| <b>Microassays</b>                               | Hybridization of DNA isolate and quantified by fluorescence based detection | Relatively high   | <60                                 | Undetermined                           | High                      | Expensive                 |
| <b>MS</b>  | Identification of heme by LDMS  | Undetermined  | Automated                           | 100 for whole blood sample             | High                      | Expensive                 |
| <b>ACC</b>                                       | Detection of malarial pigment in activated monocytes                        | Variable sensitivity and specificity                          | Automated                           | 5-20                                   | High                      | Expensive                 |

PBS: Peripheral blood smear; RDT: Rapid diagnostic technique; PCR: Polymerase chain reaction; LAMP: Loop-mediated isothermal amplification; FCM: Flow cytometric assay; MS: Mass spectrometry; ACC: Automated blood cell counters.

**Source:** Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria diagnosis: a brief review. *Korean J Parasitol*. 2009;47(2):93-102.

alone and the combination has a synergistic effect. Atovaquone inhibits mitochondrial electron transport and proguanil is a schizonticide that inhibits growth of blood and pre-erythrocytic liver stage parasites. The combination is well tolerated with infrequent, mild adverse effects. Primaquine is another drug used to treat malaria; it destroys the pre-erythrocytic hepatic stages of the parasite.<sup>15</sup> All these drugs and many more available drugs should be used wisely, to avoid increasing episodes of resistance. Furthermore, some other effective drugs like ulinastatin are also available for use in severe forms of malaria.

### Efficacy of ulinastatin

Ulinastatin is a human trypsin inhibitor that protects tissues and organs against neutrophil-mediated injury.<sup>18,19</sup> It is a glycoprotein derived from human urine. Neutrophils and their secretory products though exhibit antiparasitic activity but also induce endothelial damage that may lead to organ damage in severe malaria. A study investigated the role of ulinastatin in severe malaria. Serum samples from patients of malaria were derived and endothelial cells were cultured. Inhibition experiments were conducted, to prevent endothelial cells from neutrophil mediated apoptotic damage, ascorbic acid ( $10^{-6}$  to  $10^{-3}$  M), tocopherol ( $10^{-5}$  to  $10^{-3}$  M) and the

**Table 2: Commonly used antimalarial drugs**

| Name of the drug     | Use  | Contraindication   | Dose   |
|----------------------|--|--|--|
| Chloroquine (CQ)     | Treatment of malaria where effective and chemoprophylaxis in high risk individuals                             |  | 25 mg base/kg for 3 days   |
| Mefloquine(MQ)       | Chemoprophylaxis in CQ resistant areas and treatment of nonsevere <i>P. falciparum</i> malaria resistant to CQ | Known history of neurological or psychiatric disorders, or history of seizures | 750 to 1,500 mg base depending on local resistance pattern; prophylaxis: 250mg once per week |
| Atovaquone/Proguanil | Multiple drug resistant <i>P. falciparum</i> malaria   |  | 4 tablets of 1000 mg atovaquone and 400 mg proguanil for 3 days                              |
| Primaquine           | Treatment of <i>P. vivax</i> . gametocidal agent   | G6PD deficiency, pregnancy   | 14 mg base/day for 14 days   |
| Doxycycline          | Chemoprophylaxis and in combination with CQ to treat malaria   | Children less than 8 years, pregnant females                                   | 100mg twice daily for 3 days; prophylaxis: 100mg/ day  |
| MQ+ artesunate       | Treatment of non-severe falciparum malaria   | Same as the monotherapy  |  |

**Sources:**

1. Bloland PB, Williams HA; National Research Council (US) Committee on Population; Program on Forced Migration and Health at the Mailman School of Public Health, Columbia University. *Malaria Control during Mass Population Movements and Natural Disasters*. Washington (DC): National Academies Press; 2002. 3, Epidemiology of Malaria. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK221152/>. Accessed on: 2.8.2014.
2. Ryan ET. Malaria: epidemiology, pathogenesis, diagnosis, prevention, and treatment--an update. *Curr Clin Top Infect Dis*. 2001;21:83-113.

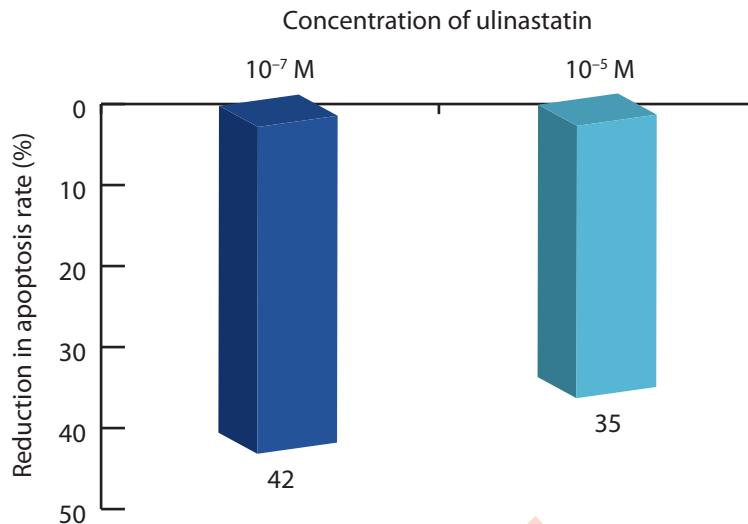
### Analogous mechanism of endothelial apoptosis in malaria and bacterial sepsis

Multiorgan failure in *Plasmodium falciparum* malaria and sepsis is attributed to apoptotic endothelial damage. Neutrophils and its secretory products play a pivotal role in malaria; they amplify endothelial apoptosis while, endothelial apoptosis is reduced by inhibitors of neutrophil-derived substances. Human umbilical vein was used as a model to study the mechanism of endothelial apoptosis in malaria and sepsis.

Endothelial cells along with patient sera or culture supernatants of malarial causative organism (*P. falciparum*) and sepsis causative organism (*Escherichia coli* and *Staphylococcus aureus*) with or without neutrophils were incubated. The reactive oxygen species or the elastase, secreted by neutrophils was neutralized by ascorbic acid or ulinastatin. The effect of direct interaction between neutrophils and endothelial cells was studied with transwell sieve inserts or antibodies against leukocyte function antigen 1 or intercellular adhesion molecule 1. TUNEL and annexin assay were used to determine the rate of apoptosis. Higher rate of endothelial apoptosis was observed in patient sera or culture supernatants with malarial and sepsis causative organisms in contrast to control sera or control supernatants. Nevertheless, the rate of apoptosis amplified on addition of neutrophils. On addition of ulinastatin and ascorbic acid, rate of apoptosis decreased in incessant presence of neutrophils. Endothelial apoptosis was also reduced when neutrophils were separated from endothelial cells with transwell sieve inserts or when anti-leukocyte function antigen-1 antibodies were introduced. Conversely, high apoptotic rate that was reduced by transwell sieve inserts was restored by addition of anti-intercellular adhesion molecule-1 antibodies.

These *in vitro* outcomes reveal a possible major contribution of neutrophils towards endothelial damage in malaria and sepsis, both by their secretory products and by binding to intercellular adhesion molecule-1 on endothelial cells. In conclusion, the existence of analogous pathognomonic mechanism suggests use of similar antiapoptotic strategies may possibly propose impeding benefits in malaria and sepsis.

**Source:** Hemmer CJ, Vogt A, Unverricht M, Krause R, Lademann M, Reisinger EC. Malaria and bacterial sepsis: similar mechanisms of endothelial apoptosis and its prevention *in vitro*. *Crit Care Med*. 2008;36(9):2562-8.

**Figure 5: Effect of ulinastatin on rate of apoptosis**

**Source:** Hemmer CJ, Lehr HA, Westphal K, Unverricht M, Kratzius M, Reisinger EC. Plasmodium falciparum Malaria: reduction of endothelial cell apoptosis in vitro. *Infect Immun*. 2005;73(3):1764-70.

serine protease inhibitor ulinastatin ( $10^{-10}$  to  $10^{-5}$  M) were added in the patient sera. Ulinastatin was chosen for its capacity to inhibit neutrophil elastase, while ascorbic acid and tocopherol were chosen for their ability to neutralize reactive oxygen species. The concentrations of these were adjusted according to levels that can be achieved by administration under clinical conditions. The results obtained revealed; ascorbic acid and urinary trypsin inhibitor ulinastatin, effectively inhibited apoptotic effect in sera of patients with severe malaria. The apoptosis rate with ulinastatin at concentration of  $10^{-7}$  M reduced to 42% and at the concentration of  $10^{-5}$  M reduced to 35% (figure 5) while no effects were observed at the concentration of  $10^{-10}$  M. After analyzing the results obtained, it was concluded that endothelial apoptosis induced by neutrophils can be prevented by use of proteolytic enzymes like ulinastatin. Thereby, may prove relevant for use in *P. falciparum* malaria as this disease is also associated with elevated levels of human neutrophils. Ulinastatin may provide clear clinical benefit in preventing organ damage due to endothelial apoptosis, although it may not clear parasite or provide symptomatic relief.<sup>20</sup>

## Conclusion

The global disease burden of malaria is increasing significantly especially in tropical and sub tropical areas of the world. Children under the age of 5 years and pregnant females are more commonly experiencing severe and fatal disease. Understanding the proper mechanism of spread of disease and the pathogenesis of disease may provide an insight to better understanding the disease. The early symptoms of the disease however being nonspecific, if diagnosed in times and backed up with laboratory diagnosis can detect the disease in its initial stages, thereby providing better control over the disease. Severe form of malaria also exists which may hamper various body system, progressing to fatal outcomes. Proper preventive and therapeutic interventions may control the disease burden. Various therapeutic options available are increasingly facing the problem of resistance. Thus, there is a need of newer agents as alternatives to undertake the problem of resistance. *In vitro* evidences show ulinastatin is a potential alternative which protects against the endothelial apoptosis in severe malaria.



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**Every year, sepsis targets around  
1 million Indians and the mortality rate  
is as high as 65%**

This World Sepsis Day take an oath to



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\*Shao Y, et al; 2005 Apr;17(4):228-30.



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