Sepsis

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Abstract

The management of the child with sepsis represents the *sine qua non* of pediatric critical care medicine. Overwhelming sepsis and septic shock often manifest with concurrent derangements of cardiovascular function, intravascular volume status, respiratory function, immune regulation, renal function, coagulation, hepatic function, and metabolic function – sepsis literally affects every organ system to some degree. The degree to which any of these derangements are manifest in a given child is highly variable and influenced by multiple host and pathogen factors, including the child's developmental stage, the presence or absence of co-morbidities, the host's immune/inflammatory state, the host's genetic background, and the specific pathogens involved. These factors combine, in turn, to profoundly influence the ultimate outcome. Successful management of critically ill children depends upon early recognition, early treatment with antibiotics, and early reversal of shock.

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Hectic fever (sepsis) at its inception is difficult to recognize but easy to treat; left untended, it becomes easy to recognize but difficult to treat…

Machiavelli, *The Prince*

Introduction

The management of the child with sepsis represents the *sine qua non* of pediatric critical care medicine. Sir William Osler once stated that *to know syphilis is to know medicine*. To a similar extent, it has been said that *to know sepsis is to know critical care medicine* [1]. Overwhelming sepsis and septic shock often manifest with concurrent derangements in cardiovascular function, intravascular volume status, respiratory function, immune/inflammatory regulation, renal function, coagulation, hepatic function, and metabolic function – sepsis literally affects every organ system to some degree. The degree to which any of these derangements are manifest in a given child is highly variable and influenced by

multiple host and pathogen factors, including the child's developmental stage, the presence or absence of co-morbidities, the host's immune/inflammatory state, the host's genetic background, and the specific pathogens involved. These factors combine, in turn, to profoundly influence the ultimate outcome.

Sepsis is a major cause of morbidity and mortality among children. Modern intensive care and a better understanding of this disease have led to significant improvements in mortality. For example, sepsis associated mortality decreased from a majority of children in 1966 (i.e., close to 100 %) [2] to approximately 10 % among children in developed countries today [3, 4]. Despite the tremendous advances made in the last few decades, the morbidity and mortality attributed to sepsis remain unacceptably high. Severe sepsis is still one of the leading causes of death in children with almost 4,500 deaths reported annually in the United States alone [5]. Worldwide, especially in developing countries, the toll of sepsis in terms of mortality and costs is probably much higher, although exact figures are lacking. However, if all of the pediatric deaths resulting from infectious diseases are considered in aggregate, sepsis is clearly the leading killer of children worldwide (see further discussion below) [4, 6].

Epidemiology of Sepsis

An accurate picture of the epidemiology of sepsis is clouded, unfortunately, by the lack of a reliable case definition [7]. However, the overall incidence of sepsis in both children and adults is now estimated at 750,000 new cases per year in the United States alone, with an overall mortality rate approaching 30 %, making sepsis the 10th leading cause of death [7–9]. Nearly 100,000 children per year present to U.S. emergency departments (EDs) with severe sepsis [10], resulting in approximately 21,000 hospitalizations [11] and 4,500 deaths in the pediatric age group [5] on an annual basis. Sepsis is the most common cause of death in infants and children worldwide – according to data from the World Health Organization (WHO), the United Nations Children's fund (UNICEF), the National Institutes of Health (NIH), and the Bill and Melinda Gates foundation, the leading killers of children during the early childhood years (pneumonia – 1.6 million/year, diarrhea – 1.3 million/year, malaria, dengue fever, influenza, and HIV) account for approximately eight million deaths a year worldwide [12]. These deaths are not officially counted as deaths attributable to sepsis, though all of these infectious diseases undoubtedly share sepsis as a common final pathway.

As noted above, extensive data regarding the epidemiology of severe sepsis during childhood have been provided by Watson and colleagues [5, 13, 14]. However, more recent studies suggest that the pediatric sepsis is becoming more prevalent [9, 15]. For example, the prevalence of severe sepsis increased by more than 80 % between 1995 and 2000. Between 2000 and 2005, the prevalence of severe sepsis increased again by 45 %. The increase in sepsis is likely due in part to the increasing life expectancy of technology-dependent children and children with chronic medical conditions. Current estimates suggest that there are between 75,000 to 100,000 cases of severe sepsis in children in the United States every year, with an associated cost of $4.8 billion [10, 15]. While the overall case fatality rate has decreased (from 1995 to 2000, the case fatality rate decreased from 10.7 to 8.9 %), because there are more children affected now, the absolute number of deaths from sepsis has increased [15].

Infants less than 1 year of age appear to be at the highest risk of sepsis, with an incidence rate that is tenfold higher than that of older children (5 cases per 1,000 in infants less than 1 year of age compared to 0.56 cases per 1,000 in all children) [5, 10, 11, 13, 14]. In this group, neonates (i.e., infants less than 28 days of age) are particularly at risk for sepsis, with prematurity and low-birth weight being significant risk factors. Again, in the series reported by Watson and colleagues, low- (LBW) and very-low birth weight (VLBW) infants accounted for nearly one-fourth of all children with severe sepsis and approximately 70 % of all infants with severe sepsis in 1995 [5]. During infancy, chronic medical conditions – especially chronic lung disease, congenital heart disease, neuromuscular disorders, and malignancies – appear to be significant risk factors. The increase in sepsis cases also reflects the growing numbers of VLBW infants in the United States. The majority of infections causing severe sepsis in children are respiratory infections (accounting for about one third of all cases) and primary bacteremias (which account for approximately one quarter of all cases of sepsis in children). Bacteremia without an apparent focus appears to be more frequent in neonates and children with an underlying malignancy, whereas respiratory infections predominate in older children without underlying illness [5, 13, 14].

Children less than 2 months of age are at significant risk for sepsis caused by group B β-hemolytic streptococcus (GBBS) and *E. coli*. Infections secondary to *Listeria monocytogenes* and the herpes simpex virus (HSV) are also prevalent in this age group. In contrast, children greater than 2 months of age are more susceptible to community-acquired organisms such as pneumococcus, *Staphylococcus aureus*, and *Neisseiria meningitidis*. Coagulase-negative staphylococci account for a significant proportion of sepsis in some series, especially in LBW and VLBW infants [5, 14, 16–18]. Meningococcal infections, by comparison, are relatively uncommon, though the case fatality rate is much higher in children with meningococcal disease (15–20 %) compared to children with pneumococcal disease (12.8–19.1 %), fungal infections (10.8–16.8 %), and sepsis secondary to coagulase-negative staphylococcal species (7.8–8.6 %).

Boys less than 10 years of age appear to be at a greater risk for sepsis [5, 11, 14]. These gender differences in both the incidence and mortality of sepsis occur in the neonatal period as well as in childhood and adolescence [19, 20], suggesting there are sex-related differences in immunity at a young age, as have been described in adults [21]. The origin of these differences in children is probably not sex hormone related, because they are most apparent at a very young age, when the influences of sex hormones are relatively unimportant.

On the basis of studies of identical twins and adoptees, genetic factors are known to be major determinants of susceptibility to death from infectious disease [22]. Some of these factors are single nucleotide polymorphisms in genes controlling the host response to microbes [23–25] and commonly used therapeutic agents [26]. Identified alterations include polymorphisms in TNF receptors, interleukin-1 receptors, Fcγ-receptor, and Toll-like receptors (TLRs). Polymorphisms in cytokine genes may determine the concentrations of inflammatory and anti-inflammatory cytokines produced during sepsis and may influence whether persons have a marked hyper inflammatory or hypo inflammatory response to infection. Combinations of polymorphisms, or haplotypes, may ultimately be used to identify patients at high risk for the development of sepsis and organ dysfunction during infection and might dictate immune based therapy to modulate the response to a given patient. The interested reader is referred to the chapter on gene polymorphisms in critical illness earlier in this textbook.

Finally, data on the long-term outcomes from sepsis are just beginning to emerge. Zimmerman and colleagues reported the long-term outcomes in survivors from pediatric sepsis in Washington state over a 14 year period (1990–2004). In their retrospective review, 7,183 children were admitted with severe sepsis, with a 28-day mortality rate of 6.8 %. An additional 6.3 % of patients died after hospital discharge. Almost half of the children who survived were re-admitted to the hospital at least once after a median of 3 months – two-thirds of these admissions were categorized as emergent [27]. Further studies like this one are desperately needed to further describe and measure the true impact of sepsis in children.

Definition of Sepsis

Bone and colleagues initially formulated a clinical definition of the *sepsis syndrome* in 1989 [28]. The constellation of signs and symptoms associated with this syndrome included elevated temperature, tachycardia, tachypnea, abnormal peripheral white blood cell (WBC) count, and evidence of organ dysfunction. Shortly thereafter, an international group of experts from the Society of Critical Care Medicine (SCCM) and the American College of Chest Physicians (ACCP) convened to refine the definition of sepsis and proposed the now familiar definitions for SIRS, sepsis, severe sepsis, and septic shock [29]. The need for a more pediatric-specific definition for SIRS, sepsis, severe sepsis, and septic shock became clear quickly, and the International Pediatric Sepsis Consensus Conference was held in 2002 to develop these definitions (Tables 30.1, 30.2 and 30.3) [31]. Because tachycardia and

Table 30.1 Definitions of systemic inflammatory response syndrome (*SIRS*), infection, sepsis, severe sepsis and septic shock

SIRS

The presence of at least two of the following four criteria, one of which must be abnormal temperature or leukocyte count

    Core temperature of >38.5 °C or <36 °C (by rectal, bladder, oral or central catheter probe)

    Tachycardia defined as mean heart rate >2SD for age[a] or otherwise persistent elevation over a 0.5–4 h time period (see Table 30.2 for age-specific ranges)

    Or for children <1 year old: bradycardia, defined as a mean heart rate <10th percentile for age[b] or otherwise persistent depression over a 0.5 h period

    Mean respiratory rate >2 SD above normal for age or mechanical ventilation[c]

Infection

A suspected or proven infection caused by any pathogen OR a clinical syndrome associated with a high probability of infection. Evidence of infection includes positive findings on clinical examination, imaging or laboratory tests[d]

Sepsis

SIRS in the presence of or as a result of suspected or proven infection

Severe sepsis

Sepsis plus one of the following: cardiovascular organ dysfunction OR acute respiratory distress syndrome OR two or more organ dysfunctions (as defined in Table 30.3)

Septic shock

Sepsis and cardiovascular organ dysfunction as defined in Table 30.3

[a]In the absence of external stimulus, chronic drugs, painful stimuli
[b]In the absence of external vagal stimulus, β-blockers or congenital heart disease
[c]Not for underlying neuromuscular disease or receipt of general anesthesia
[d]By positive culture, tissue stain, or polymerase chain reaction

Table 30.2 Age specific vital signs and laboratory variables

Age group	Heart rate (beats/min) tachy	brady	Respirations (breaths/min)	Leukocyte count (×10³/mm³)	Systolic blood pressure (mm Hg)
0 days – 1 week	>180	<100	>50	>34	<65
1 week – 1 month	>180	<100	>40	>19.5 or <5	<75
1 month – 1 year	>180	<90	>34	>17.5 or <5	<100
2–5 year	>140	NA[a]	>22	>15.5 or <6	<94
6–12 year	>130	NA	>18	>13.5 or <4.5	<105
13–18 year	>110	NA	>14	>11 or <4.5	<117

Lower values for heart rate, leukocyte count and systolic blood pressure are for the 5th and upper values for heart rate, respiration rate, and leukocyte count for the 95th percentile

[a]NA = not applicable

Table 30.3 Organ dysfunction criteria

Cardiovascular dysfunction

Despite the administration of isotonic intravenous fluid bolus ≥40 mL/kg in 1 h

    Decrease in BP ≤5th percentile for age OR

    Need for vasoactive drug to maintain BP in the normal range* OR

    Two of the following

    Unexplained metabolic acidosis: base deficit >5.0 mEq/L

    Increased arterial lactate >2 times upper limit of normal

    Oliguria: urine output <0.5 ml/kg/h

    Prolonged capillary refill: >5 s

    Core-peripheral temperature gap >3 °C

Respiratory

    $PaO_2/FIO_2 < 300$ in the absence of cyanotic heart disease or preexisting lung disease OR

    $PaCO_2 > 20$ mmHg or 2,7 kPa over baseline $PaCO_2$ OR

    Proven need for >50 % FIO_2 to maintain saturation >92 % OR

    Need for non-elective invasive or noninvasive mechanical ventilation

Neurological

    Glasgow coma scale ≤11 OR

    Acute change in mental status with decrease in Glasgow coma scale ≥3 points from baseline.

Hematological

    Platelet count <100.000/mm³ or a decline of 50 % in platelet count from highest value recorded over the last 3 days OR

    International normalized ration >2

Renal

    Serum creatinine ≥2 times the upper limit of normal for age or a twofold increase in baseline creatinine

Hepatic

    Total bilirubin ≥4 mg/dL (not applicable for the newborn) OR

    ALT 2 times the upper limit of normal

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tachypnea are common symptoms in a variety of pediatric conditions, the major difference in the definition of SIRS between children and adults is that the diagnosis of pediatric SIRS requires that temperature or leukocyte abnormalities are also present. In children less than 1 year of age, bradycardia may be a sign of SIRS and subsequently this condition is also included in the definition. Finally, the presence of fever is defined as >38.5 °C, which provides a more specific cut off than 38 °C. The definitions propose six age groups for age-specific vital sign and laboratory parameters: newborn (0–1 week), neonate (1 week to 1 month), infant (1 month to 1 year), toddler and preschool (2–5 years), school age child

(6–12 years), and adolescent and young adult (13–18 years). These categories are based on expert opinion, since evidence based values for abnormal vital signs and laboratory markers are lacking [32]. As new insights develop, the definition of sepsis will also need to be re-evaluated.

The validity and usefulness of the consensus sepsis definitions has been widely debated in the literature due to its relative non-specific qualities and the broad range of patients that could be classified as having SIRS [32–35]. For example, Proulx and colleagues analyzed the incidence and outcome of SIRS, sepsis, severe sepsis, and septic shock in a single tertiary care children's hospital [36]. Out of 1,058 admissions

Table 30.4 Factors determining the severity of sepsis according to the PIRO concept: Predisposition, Infection, Response and Organ dysfunction

Predisposing factors
Pre-morbid conditions like cancer, primary or secondary immunodeficiencies, cystic fibrosis sickle cell disease and asplenia
Gender and age of the patient: e.g., children <2 year are more susceptible to encapsulated bacteria
Genetic factors: gene polymorphisms in cytokine genes or other immune effector molecules
Presence of immunity against certain micro-organisms (vaccination status, previous infections)
Medication: immune-suppressive agents, cardiovascular drugs
Nutritional status: malnutrition is a state of immunodeficiency
Trauma or surgery; increased risk of infection
Presence of foreign substances such as intravenous catheters
Infection
Differences in virulence factors of the causative microbe such as production of LPS, causes different pathology
Size of the inoculum
Initial site of infection: influences the chance of generalization of the infection
Antimicrobial resistance of the pathogen: influence on response on initial antibiotic therapy
The time to recognition of infection by clinical judgment and appropriate laboratory and imaging studies affects outcome
Response of the patient
The host response is determined by the predisposing factors: the nature of the host response influences the chances of survival and significant morbidity: diffuse intravascular coagulation, balance between of pro- and anti-inflammatory cytokines
This response is modified by timely interventions of the physician, such as antibiotics and early goal directed therapy, determining the chance of the development of organ dysfunction
Organ dysfunction
Variations in patterns of organ dysfunction like myocardial depression, ARDS, renal failure and liver failure affect outcome. They are the result of the complex interaction between microcirculatory dysregulation, energy depletion and apoptosis. The severity of organ dysfunction is the major determinant of the outcome of sepsis

analyzed over a 1-year period, 82 % of children admitted to the pediatric intensive care unit (PICU) met criteria for SIRS, while 23 % had sepsis, 4 % had severe sepsis, and 2 % had septic shock. The SIRS criteria also lack sufficient sensitivity and specificity outside the PICU environment, where early recognition and diagnosis are critically important. For many of these reasons, the "PIRO" concept [37] was proposed as a staging system for sepsis, modeled after the TNM (**T**umor, **N**odes, **M**etastasis) system [38] for staging malignancies. The PIRO staging system stratifies patients on the basis of their **P**redisposing conditions, the nature and extent of the insult (**I**nfection), the nature and magnitude of the host **R**esponse, and the degree of concomitant **O**rgan dysfunction (Table 30.4). The PIRO staging system has many favorable attributes, but will require thorough validation and testing before it is widely adopted and applied in clinical practice [39–41], particularly in pediatrics [42, 43].

Importantly, although sepsis is defined as a systemic inflammatory response to an inciting infection, an infectious microorganism is isolated in fewer than 50 % of cases [44]. A suspected infection, as shown by positive findings at physical examination (e.g., signs of arthritis or osteomyelitis), petechial rash, or abnormalities on auscultation of the chest can therefore define *sepsis*. Laboratory evidence of infection such as the presence of white blood cells in a normally sterile body fluid or imaging techniques showing pulmonary infiltrates can also validate the suspicion of an infection. It should

also be noted that in the definition of *septic shock*, there is no requirement for systemic hypotension, because children are often able to maintain their blood pressure at the expense of peripheral perfusion, even if they are severely ill.

Scoring systems have been developed that attempt to quantify the degree of organ dysfunction or failure in critically ill children, especially in those children with severe sepsis. For example, the number of dysfunctional organs is frequently used as a surrogate marker of disease severity in children with MODS, with the rational that the greater the number of dysfunctional organs the worse the outcome. This score is called the pediatric MODS score and has been used by several investigators [45]. The Pediatric Logistic Organ Dysfunction (PELOD) score has recently been validated as an outcome measure in children with MODS [46]. In addition, Graciano and colleagues recently developed and prospectively evaluated the Pediatric Multiple Organ Dysfunction Score (P-MODS) using a database of 6,456 consecutive admissions to the PICU at their tertiary care institution [47]. Finally, there are a number of disease-specific scoring systems that have been proposed for use in children with meningococcemia and septic shock [48–51]. Scoring systems such as these are important to link the severity of sepsis with measured outcome variables. Further information regarding these types of scores is provided in other chapters of this textbook. In addition, recent work by our group suggests that a pediatric sepsis biomarker risk profile

(PERSEVERE) reliably identifies critically ill children at risk of death from septic shock. PERSEVERE, which is based upon five biomarkers performs better than any of the currently used severity of illness scores [52].

Clinical Manifestations

It cannot be over-emphasized that the early recognition of sepsis is the key to successful management [53, 54]. Pediatricians need to be constantly aware of the possibility of infections and subsequent sepsis in children admitted to the PICU. A possible focus of infection should be elicited by careful history and physical examination. All the major organ systems of the body are adversely affected by inadequate oxygen delivery from perturbations in cardiac and respiratory function. In addition, these organ systems are also injured by the direct cytotoxic effects of bacterial toxins and circulating cytokines (see below).

The onset of sepsis is often insidious and the initial signs and symptoms are therefore relatively non-specific and reflect the body's attempt to compensate for poor oxygen delivery. Children often present with fever, tachypnea (reflecting a compensatory mechanism aimed at counteracting the metabolic acidosis which occurs due to inadequate oxygen delivery to the tissues), tachycardia (reflecting a compensatory mechanism to increase cardiac output and oxygen delivery to the tissues), leukocytosis or leukopenia, thrombocytopenia, and alterations in mental status. Fever is frequently the initial manifestation of infection and is believed to result from the release of a number of the cytokines elicited in response to infection, including tumor necrosis factor (TNF)-α and interleukin (IL)-1β. Children, especially infants, may also present with hypothermia, which appears to be associated with worse outcome in adults with sepsis [55]. The mental status of a child with sepsis is frequently altered, ranging from agitation to obtundation. This depressed mental status can be present even in the absence of meningitis and reflects inadequate oxygen delivery to the central nervous system. The skin is frequently hypoperfused with decreased capillary refill and mottling. Petechiae and purpura are sometimes present and, while helpful in diagnosis, can be ominous signs. For example, petechiae classically points towards meningococcal sepsis, but can also be manifest in several other infections (e.g., *Haemophilus influenzae*, *Streptococcus pneumonia*, *Staphylococcus aureus*, enterovirus, herpes simplex virus, varicella, etc.).

The four major categories of shock proposed in a classification scheme by Hinshaw and Cox [56] in 1972 include: (i) hypovolemic shock (shock as a consequence of inadequate circulating volume), (ii) obstructive shock (shock caused by obstruction of blood flow to and from the heart), (iii) cardiogenic shock (shock caused by primary pump failure), and (iv) distributive shock (shock caused by maldistribution of the circulating volume). Septic shock does not readily fall into any one of these four categories. Rather, septic shock is unique in that several of these forms may be present simultaneously in the same affected child. For example, a child with septic shock may exhibit signs and symptoms consistent with hypovolemic shock secondary to capillary leak, increased insensible losses, and decreased effective blood volume secondary to venodilation; cardiogenic shock secondary to the myocardial-depressant effects of bacterial toxins and inflammatory cytokines; and distributive shock from decreased systemic vascular resistance. The degree to which any one child manifests these physiologic perturbations varies considerably. For example, Ceneviva et al. [57] categorized 50 children with fluid-refractory septic shock based upon hemodynamic data obtained with the pulmonary artery (PA) catheter into one of three possible cardiovascular derangements – (i) a hyperdynamic state characterized by a high cardiac output (>5.5 L/min/m^2 BSA) and low systemic vascular resistance (<800 dyn sec/cm^5) (classically referred to as *warm shock*); (ii) a hypodynamic state characterized by low cardiac output (<3.3 L/min/m^2 BSA) and low systemic vascular resistance (SVR); or (iii) a hypodynamic state characterized by low cardiac output and high SVR (>1,200 dyn sec-cm^5) (classically referred to as *cold shock*). In stark contrast to adults in which the early stage of septic shock is most often (~90 %) characterized by a high cardiac output and low SVR (*warm shock*), most of the studied children (~60 % of cases) were in a hypodynamic state characterized by low cardiac output and high systemic vascular resistance (*cold shock*) and required the addition of inotropes and vasodilators to decrease SVR, increase CI, and improve peripheral perfusion [57]. Children with low cardiac output (as defined by a cardiac index less than 2.0 L/min/m^2 BSA) had the highest risk of mortality. These findings have been confirmed in multiple studies using a variety of methods to measure cardiac output and vascular resistance [58–64]. Collectively, these studies all point to the fact that hypotension in children is a very late sign that portends a poor prognosis. Moreover, children more commonly present with cold shock, as opposed to warm shock.

The Immunopathobiology of Sepsis

Advances in molecular immunology methods and the use of genetically modified animal models have uncovered remarkable complexity in the pathophysiology of sepsis. An in-depth understanding of the dynamic host immune response to sepsis is a critical antecedent for successful clinical intervention. The addition of immune biomarker-based staging of disease to clinical sign staging is highly likely to increase the accuracy of patient classification for future multi-site clinical trials that will test novel interventions. Lastly, the host

immune response to sepsis is strongly determined by developmental age [65, 66]. As we have emphasized repeatedly, children are not small adults and experimental findings from adult studies should not be assumed to apply to children and neonates.

Sepsis is, by definition, a systemic inflammatory response associated with infection in a normally sterile area [67]. An immune response to eradicate any infectious challenge is appropriate and necessary to prevent evolution and spread of the pathogen throughout the host. However, in some cases the inflammation is not limited and becomes generalized, resulting in the constellation of signs and symptoms of SIRS, as described above. SIRS may or may not be associated with infection. If the infection is not contained, the spread of the pathogen through the blood may result in systemic endothelium activation and precipitate severe sepsis and septic shock. Host immunity is grossly divided into innate and adaptive responses, but in reality there is a great deal of cross-talk between the two systems. Innate immunity is rapid, largely non-specific, and is composed of barriers, phagocytic cells, the complement system, and other soluble components of inflammation. Following breech of a barrier, cellular elements of the innate immune response are the first line of defense against the development and progression of infection. Adaptive immunity, which is antigen-specific, long-lived, and takes several days to develop, provides immunologic specificity and memory. Both systems play important roles in the pathophysiology of sepsis.

Barrier Defenses

Barriers, including skin and mucosal surfaces, are the primary immune defense against the development of local infection. Barrier function is critical because these areas represent the first point of contact between the host and potential pathogens. Multiple immune elements are present to prevent attachment and propagation of pathogens, while simultaneously permitting the presence of commensal organisms required for homeostasis. In contrast to the moist mucosal surfaces of the respiratory and gastrointestinal tracts, the skin is arid which reduces the chances for microbial invasion. The outer layer of skin, the *stratum corneum*, is covered with antimicrobial peptides that possess microbicidal activity [68]. Disruption of the cutaneous barrier via trauma or burn allows microorganisms to enter the subcutaneous tissue increasing the likelihood of establishing a local infection.

Mucosal barriers are defended by several components that serve to prevent invasion including mucus, cilia, antimicrobial peptides, soluble opsonins, destructive enzymes, acidic pH, commensal organisms, and sentinel immune cells such as macrophages, dendritic cells (DCs), neutrophil or polymorphonuclear cell (PMNs), and T cells. The respiratory mucosa is defended by epithelial cells, cough, mucociliary clearance, resident professional phagocytes, and the secretion of a number of proteins and peptides with host defense functions. The collectins, surfactant protein A and D, possess valuable immune function by increasing opsonization of inhaled pathogens. Respiratory mucosal function can be disrupted through altered mucus production, intubation and mechanical ventilation (decreased mucociliary clearance, increased mucus production, and airway irritation), surfactant deficiency, and physical damage to lung parenchyma (volutrauma, atelectotrauma, barotrauma, chemical injury, or infection).

Gastrointestinal (GI) barrier homeostasis may be disrupted by infection or chemotherapy that leads to mucositis. GI barrier integrity is also dependent on the interaction between commensal organisms and host epithelium. Alteration of this relationship with antibiotics or stress in the form of hypoxia or remote infection may increase the risk for barrier dysfunction and bacterial translocation. Under these circumstances, the gut may become the "*motor of systemic inflammation*" [69]. Therefore, maintenance of GI barrier integrity is paramount for prevention of spread of microorganisms out of the mucosal compartment.

Molecular Events During Early Infection

Pathogen Recognition

Once the local barrier function has been compromised, pathogen recognition by local immune sentinel cells is the first step towards the development of an immune response (Figs. 30.1 and 30.2). Pattern recognition receptors (PRRs) [71] including Toll-like receptors (TLRs) facilitate recognition of pathogen-associated molecular patterns (PAMPs) [71]. A litany of PAMP-sensing PRR classes exist including the TLRs, Nod-like receptors (NLRs), retinoic-acid-inducible protein I (RIG-I)-like receptors (RLRs), peptidoglycan recognition proteins (PGRPs), β-integrins, and c-type lectin receptors. The discovery that TLR4 was integral for a robust lipopolysaccharide (LPS)-mediated inflammatory response that occurs with gram-negative sepsis may be why TLRs have been more thoroughly investigated in the setting of sepsis than other PRRs.

Each of the ten known TLRs in humans, present on and within multiple cell types, recognizes extracellular and intracellular pathogens via specific PAMPs [72, 73]. Multiple TLRs may be activated in concert by intact or partial microorganisms and activate multiple second messenger pathways simultaneously [73, 74]. LPS in the gram-negative bacterial cell membrane is the archetype PAMP and a key mediator of systemic inflammation, septic shock, and multi-organ failure and death [75]. LPS signals primarily through TLR4 in conjunction with the cell surface adaptor proteins CD14 and
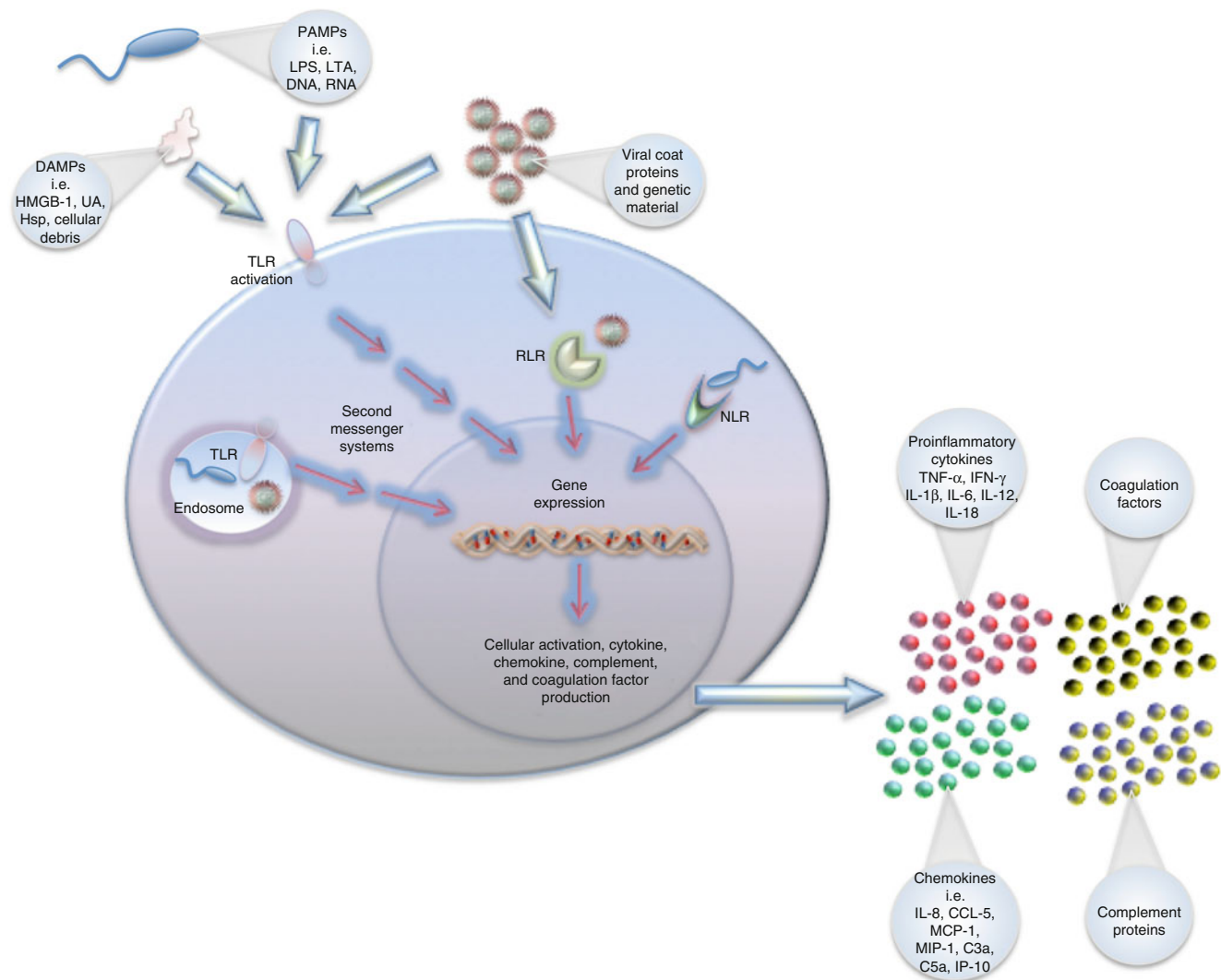
Fig. 30.1 Activation of sentinel immune cells. Sentinel cells (e.g., monocyte, macrophage) sense pathogens via PAMPs or DAMPs binding to PRRs. Pathogen recognition receptors (*PRRs*) include *TLRs* Toll-like receptor, *RLRs* Rig-1-like receptors, and *NLRs* NOD-like receptors. Pathogen associated molecular patterns (*PAMPs*) include *LPS* lipopolysaccharide, *LTA* lipotechoic acid, DNA, and RNA. Damage/Danger associated molecular patterns (*DAMPs*) can also be sensed through TLRs and include uric acid (*UA*), heat shock proteins (*Hsp*), and HMGB-1. Signaling occurs through a series of second messengers and results in transcription and translation of cytokines and chemokines that amplify the immune response (Reprinted with permission from Wynn and Wong [70]. With permission from Elsevier)

MD2 [71]. Bacterial cell wall components (such as lipoteichoic acid) signal primarily through TLR1/2/6, flagellin through TLR5, and CpG double-stranded DNA through TLR9. Common viral PAMPs such as double-stranded RNA or single-stranded RNA signal through TLR3 and TLR7/8 respectively.

Agonist-TLR binding results in a signaling cascade of intracellular second messenger proteins ultimately leading to production of cytokines and chemokines as well as activation of other antimicrobial effector mechanisms [72]. Key intracellular messengers critical for effective TLR signaling include myeloid differentiation factor 88 (MyD88), toll-interleukin (IL)-1 receptor domain containing adaptor protein (TIRAP), Toll/IL-1 receptor homology (TIR) domain containing adapter-inducing interferon B (TRIF), TRIF-related adaptor molecule (TRAM), IL-1 Receptor-Associated Kinase (IRAK)-1 and IRAK-4, and nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKBIA). Signaling through MyD88 typically leads to the production of nuclear factor kappa B (NF-κB)-dependent inflammatory cytokines/chemokines, whereas signaling through TRIF induces production of type I interferons as well as NF-κB-related inflammatory cytokines.

Because TLRs play an essential role in recognition and response to pathogens, alterations in their expression, structure, signaling pathways, and function can have consequences
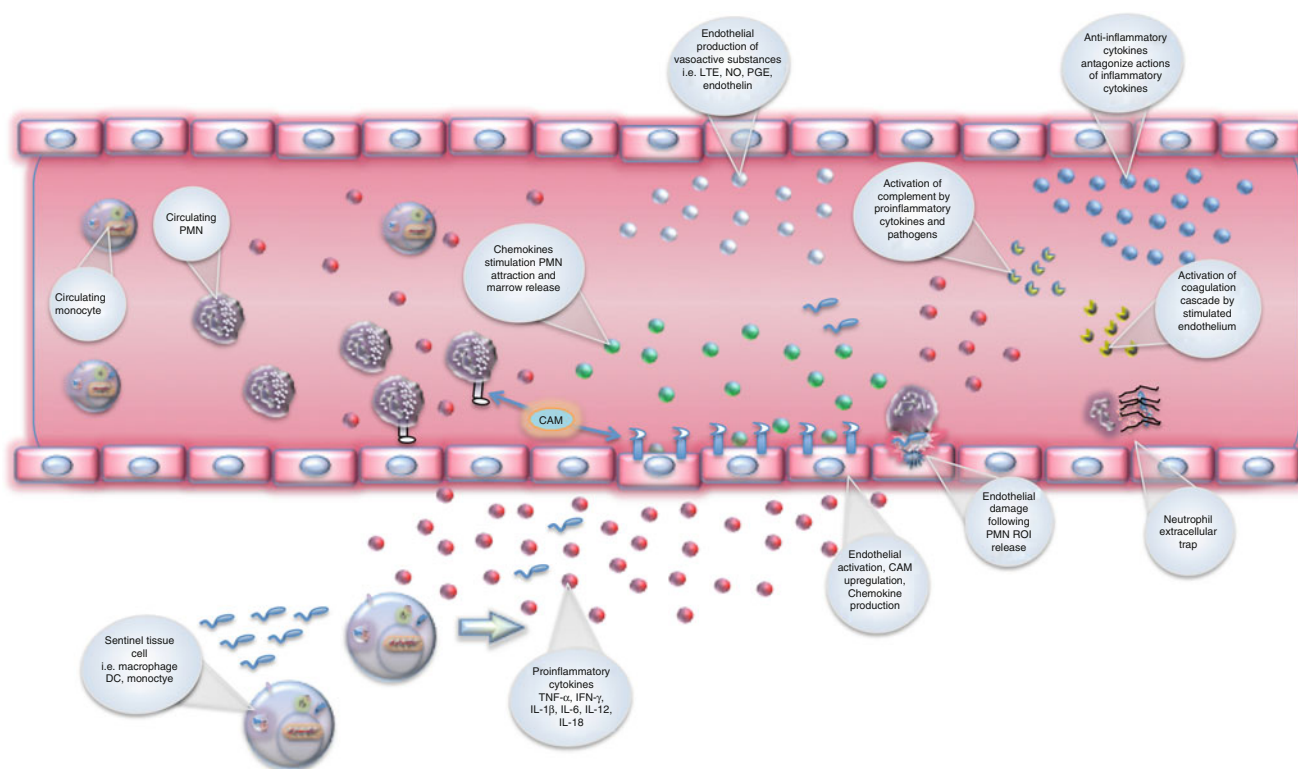
Fig. 30.2 Cellular recruitment and endothelial activation following pathogen detection. Pathogen-stimulated tissue/blood monocytes, dendritic cells (*DC*), and macrophages release proinflammatory cytokines that activate the surrounding endothelium. Endothelial activation results in upregulation of cell adhesion molecules (*CAM*), production of chemokines and vasoactive substances, activation of complement, and development of a procoagulant state. Recruitment of PMNs occurs along the chemokine gradient surrounding the area of inflammation. Anti-inflammatory cytokines counter the actions of proinflammatory cytokines to prevent excessive cellular activation and recruitment that can result in tissue damage and systemic inflammation. Endothelium can be damaged when PMNs release reactive oxygen intermediates (*ROI*) or form NETs. LTE-leukotriene, NO-nitric oxide, PMN-neutrophil. NET-Neutrophil extracellular trap (Reprinted with permission from Wynn and Wong [70]. With permission from Elsevier)

to host defense. Polymorphisms or mutations in TLRs are associated with increased risk for infection in both adults [76–79] and children [80–82]. Modifications in expression or function of co-stimulatory molecules necessary for TLR activation are also associated with an increased risk for infection. Genetic variations in CD14 (LPS co-receptor) and LPS binding protein have been associated with increased risk for sepsis in adults [83–85]. Gene polymorphisms in myeloid differentiation-2 (MD-2), a small protein involved in LPS signaling through TLR4, increase the risk for organ dysfunction and sepsis in adults [86], but the significance in children is unknown. Polymorphisms in select cytokines (IL-6 and IL-10) or their receptors [87] and constituents of their signaling pathways may be associated with increased risk of infection [88–91], though there is not complete agreement on these findings [92–94]. Polymorphisms in downstream (post-TLR activation) intracellular signaling molecules including MyD88 [95], IRAK-4 [96], and NF-κB essential modulator (NEMO) [97] are associated with invasive bacterial infection in older populations.

Damage-associated molecular patterns (DAMPs), such as intracellular proteins or mediators released by dying or damaged cells, may also active PRRs. For example, the DAMP High mobility group box-1 (HMGB-1) is involved in the progression of sepsis to septic shock [75, 98]. HMGB-1 is produced by macrophages or endothelial cells stimulated with LPS or TNF-α and signals through TLR2, TLR4, and receptor for advanced glycation end products (RAGE) [99]. HMGB-1 results in cytokine production, activation of coagulation, and PMN recruitment [98, 100]. HMGB-1 mediates disruption of epithelial junctions within the gut via the induction of reactive nitrogen intermediates (RNI), leading to increased bacterial translocation [101]. Other specific DAMPs, including heat shock proteins (Hsps) and uric acid, may also stimulate TLRs, regulate PMN function, and function as immune adjuvants. Hsp are significantly elevated in septic adults and children [102]. Elevated Hsp60 and Hsp70 measured within 24 h of PICU admission was associated with pediatric septic shock and there was a strong trend towards a significant association with death [103, 104]. Cytokine production, PMN recruitment, and
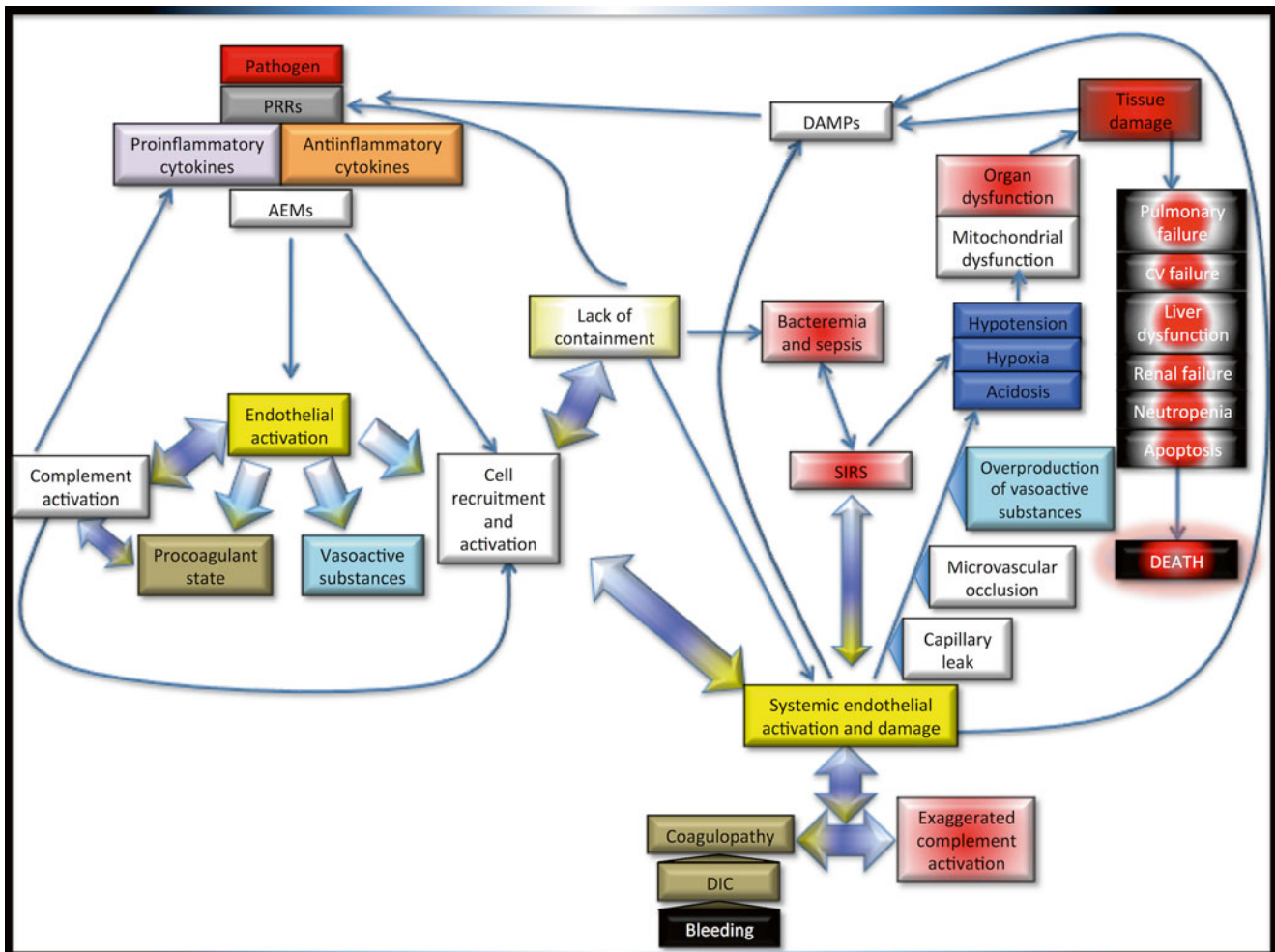
Fig. 30.3 Pathophysiology of sepsis and septic shock. *PRR* Pattern recognition receptors, *AEM* antimicrobial effector mechanisms, *DAMP* Danger/damage-associated molecular patterns, *SIRS* Systemic inflammatory response syndrome, *DIC* disseminated intravascular coagulation, *CV* cardiovascular (Reprinted with permission from Wynn and Wong [70]. With permission from Elsevier)

dendritic cell stimulation [105] are all increased following exposure to uric acid. In addition to its role as a pro-inflammatory DAMP, uric acid may also serve as an potentially beneficial antioxidant [106].

Inflammatory Mediator Production

PRR stimulation results in rapid cytokine and chemokine production alongside amplification of the innate cellular response directed at cellular activation and clearance of pathogenic organisms. Pro-inflammatory cytokines including IL-1β, IL-6, CXCL8 (IL-8), IL-12, IL-18, interferon gamma (IFN-γ), and TNF-α are produced early in the host response and activate innate immune effector cells [107]. Compared to septic adults, septic neonates produce less IL-1β, TNF-α, IFN-γ, and IL-12 [108–113]. A wide variety of chemokines are increased during sepsis, including CXCL10 (IP-10), CCL5 (RANTES), CCL2 (MCP-1), CCL3 (MIP-1a), and CXCL8 (IL-8) [114]. Other chemo-attractive molecules also increase with sepsis, including

complement proteins C3a and C5a, antimicrobial proteins or peptides including cathelicidins and defensins, as well as components of invading bacteria themselves [107, 115]. The importance of chemo-attractive substances in the pathogenesis of severe sepsis is highlighted by recent studies showing that CXCL8 can be used a stratifying factor for survival in children [116], and C5a is implicated in sepsis-associated organ dysfunction in adults [75]. Chemokine investigations in septic neonates revealed that CXCL10 is a sensitive early marker of infection [114], and low CCL5 levels may predict development of DIC [117].

Careful interplay between anti- and pro-inflammatory stimuli serves to govern the immune response to allow local pathogen containment but prevent systemic activation leading to excessive inflammatory damage though SIRS associated with multi-organ failure and death (Fig. 30.3) [118]. The previously accepted host-response to sepsis paradigm (an intense pro-inflammatory response, or SIRS temporally

followed by a compensatory anti-inflammatory response syndrome [CARS]) has been challenged by the failure of multiple anti-inflammatory strategies. New data demonstrate a simultaneous pro/anti-inflammatory response where the magnitude may determine outcome [119]. Near simultaneous increases in anti-inflammatory cytokine production (TGF-β, IL-4, IL-10, IL-11, and IL-13) occur during infection countering the actions of pro-inflammatory cytokines [107, 120, 121]. These mediators blunt the activation of phagocytic cells, block fever, modify coagulation factor expression, and decrease production of ROI/RNI, NO, and other vasoactive mediators [122–126].

Soluble cytokine and receptor antagonists produced during sepsis also modulate pro-inflammatory mediator action. Elevation of TNFR2 (which regulates the concentration of TNF-α), sIL-6R, sIL2, and IL-1ra have been documented in neonatal sepsis with resolution following effective treatment [121, 127, 128]. Soluble RAGE (sRAGE) competes with cell-bound RAGE for the binding of HMGB-1 and other RAGE ligands [129]. sRAGE has anti-inflammatory effects, is elevated in adults during sepsis [130], improved survival and reduced inflammation when given to infected adult rodents [131].

Complement

Complement facilitates killing of bacteria through opsonization and direct microbicidal activity. Complement components also possess chemotactic or anaphylactic activity that increases leukocyte aggregation and local vascular permeability at the site of invasion. Furthermore, complement reciprocally activates a number of other important processes such as coagulation, proinflammatory cytokine production, and leukocyte activation [75]. Contrary to its name, the *alternative* pathway is actually the primary mechanism of amplification of complement activation following C3 convertase assembly (which cleaves C3 to C3a and C3b) formation via the classical, lectin, or alternative pathways. Dysregulation of complement activation may participate in the untoward effects with severe sepsis or septic shock. Importantly, neonates, particularly the very premature, exhibit decreased basal levels of complement proteins and function for both the alternative and classic pathways [132, 133].

C3b and C5a facilitate opsonization (primarily C3b), redistribution of blood flow, increased inflammation, platelet aggregation, and release of ROI (primarily C5a) [134, 135]. C5a-mediated local leukocyte activation also results in increased cytokine production with subsequent upregulation of adhesion molecules on vascular endothelium allowing for increased cell recruitment to the site of infection [136]. Deficiencies in C5aR found in term neonates as compared to adults may limit the ability to respond to C5a and therefore increase the likelihood of infection [137].

Complement regulatory proteins (CD55, CD59) modify the effects of complement and prevent potential damage due to over-activation [138]. Dysregulation of complement activation can lead to a vicious activation cycle that results in excessive cellular stimulation, cytokine production, endothelial cell activation, and local tissue damage. Dysregulation likely contributes to the development of SIRS and shock [139]. Elevated C5a levels are associated with the development of DIC, via increased tissue factor expression, cardiomyopathy, increased pro-inflammatory cytokine levels and the development of SIRS, adrenal insufficiency, and PMN dysfunction [75].

In addition to the initial inflammatory response and complement activation following pathogen recognition, infection results in increases in multiple other innate proteins that possess valuable immune function [140]. These components serve to reduce bacterial load and include collectins (e.g., surfactant proteins A and D), lactoferrin, cathelicidin, bacteriocidal permeability increasing protein (BPI), and phospholipase A_2 [141]. Acute phase reactant proteins including CRP (opsonin), lactoferrin (reduce available iron/antimicrobial peptide-lactoferricin), serum amyloid A (cellular recruitment), procalcitonin (unknown function), haptoglobin, fibronectin (opsonic function), pentraxin 3 (binds C1q and activates classical complement pathway) and others increase significantly during sepsis and provide useful adjuvant immune functions [107]. Natural antibodies (predominantly IgM) produced by circulating B1 lymphocytes augment opsonizing capacity [142–144]. PMNs from term neonates are deficient in BPI, potentially contributing to the increased risk for infection [145]. Polymorphisms in BPI increase the risk for gram-negative sepsis in children [146]. Mannose-binding lectin (MBL) is capable of activating the lectin and alternative complement pathways, and decreased MBL levels are associated with an increased risk of sepsis during the first month of life [147, 148]. Despite increases in acute phase and other innate proteins with infection, neonatal plasma has significantly impaired opsonizing activity compared to adults that increases the likelihood of progression to systemic infection [149].

Coagulation Cascade

An important part of host pathogen containment includes the development of a pro-coagulant state in the microvasculature surrounding a focal site of infection. However, if systemic endothelial activation occurs or proinflammatory cytokine production is high, over-activation of the coagulation system may occur and lead to DIC, resulting in severe tissue and organ damage [150]. In general, the intrinsic pathway amplifies coagulation following initiation by the extrinsic pathway [151]. Important differences exist in children as compared to adults in the coagulation system. Reduced vitamin K-dependent factors (Factor II, VII, IX, X), thrombin generation, consumption of platelets with formation of microthrombi, and counter-regulatory elements (inhibitors) increase the risk for bleeding in pediatric patients [152].

Coagulation cascades during infection may begin with cytokine-activated PMNs, monocytes, or endothelium, which express increased tissue factor apoprotein [153, 154]. Activation of tissue factor leads to increased clotting proteins including thrombin-antithrombin complex (TAT), plasminogen activator inhibitor (PAI), and plasmin-a2-antiplasmin complex [155]. There is also a shift towards inactivation of protein S and depletion of anticoagulant proteins including antithrombin III (ATIII) and protein C [156, 157]. Cytokine production increases expression of endothelial tissue plasminogen activator inhibitor type-1 (PAI-1). PAI-1 inhibits fibrinolysis by inhibiting the conversion of plasminogen to plasmin important for the breakdown of fibrin. Deposition of fibrin in small vessels leads to inadequate tissue perfusion and organ failure [158]. Increased PAI-1 activity levels are associated with increasing IL-6, nitrite and nitrate levels, the development of organ failure (cardiovascular, renal, hepatic, coagulatopathy), and mortality [158].

Clotting can lead to propagation of inflammation via thrombin-induced production of platelet-activating factor (PAF). PAF-activated or platelet TLR4-activated PMNs may then contribute to further endothelial injury and dysfunction leading to the development of a vicious clotting-inflammation-clotting cycle. The protease activated receptor (PAR)-1 also plays a major role in orchestrating the interplay between coagulation and inflammation [159]. PAR-1 may modify the endothelial response during pediatric sepsis and thus represent another target for therapeutic intervention.

Innate Immune Cellular Contributions

PMN

The PMN is the primary effector of innate immune cellular defense. Endothelial cells produce activating cytokines and chemokine gradients that recruit circulating PMNs to the site of infection. Expression of cell adhesion molecules by PMNs and endothelium allows cells to roll and extravasate into surrounding tissues. Activated PMNs phagocytose and kill pathogens via oxygen-dependent and oxygen-independent mechanisms. IL1β is produced by activated PMNs largely via an NLRP3/ASC/CASP1-dependent mechanism that amplifies the recruitment of additional PMNs from the bone marrow to the site of infection [160]. Activated PMNs may release RNI, reactive oxygen intermediates (ROI) and proteolytic enzymes extracellularly via activation of membrane associated-NADPH oxidase. These reactive intermediates and enzymes can lead to destruction of non-phagocytized bacteria but also can cause local tissue destruction [161, 162] and thus play a role in progression from sepsis to multi-organ dysfunction syndrome (MODS). With PMN death, the DNA (chromatin), histone and antimicrobial proteins are expelled into the environment and serve to trap bacteria (neutrophil extracelluar traps, NETs) [163]. The formation of NETs can occur following activation of platelet TLR4 [164] and may lead to excessive local inflammation and tissue damage [165]. High early levels of circulating free neutrophil-derived DNA produced by NETs are associated with multiple organ failure, and death [166].

PMNs exhibit quantitative and qualitative differences related to developmental age [167, 168]. Rapid depletion of bone marrow PMN reserves during infection, particularly in neonates [169], can lead to neutropenia with consequent impaired antimicrobial defenses and significantly increased risk for death [170]. Neutropenia is particularly common in gram-negative sepsis in neonates [171]. PMN respiratory burst activity may also suppressed during sepsis and may contribute to poor microbicidal activity [172–174]. Release of immature PMN forms (bands), which have greater dysfunction than mature PMNs [175], may further predispose to adverse outcomes.

Antigen-Presenting Cells (APCs)

The role that APCs play in the host immune response and pathophysiology of sepsis is incompletely characterized. Monocytes, macrophages, and dendritic cells amplify cellular recruitment through production of inflammatory mediators and activation of endothelium, phagocytosis and killing of pathogens, and antigen presentation to T and B cells of the adaptive immune system. DCs are "professional APCs" and are depleted from the spleen and lymph nodes with sepsis in animal models and may be important for survival [176]. Monocytes and macrophages are closely related to PMNs (common myeloid progenitor) and can kill pathogens by similar means. Circulating monocytes differentiate into macrophages following exposure to maturing cytokines and exit the blood stream into tissues. Important substances produced by stimulated monocytes/macrophages that may contribute to sepsis and septic shock include complement components, cytokines (both pro and anti-inflammatory), coagulation factors, and extracellular matrix proteins [177].

Vascular Endothelium

Recent studies have shown the critical importance of vascular endothelial activation in the early recognition and containment of microbial invasion. Expression of TLRs allows endothelium to become activated in the presence of microbial components, leading to production of cytokines and chemokine gradients, as well as adhesion molecules (VCAM, ICAM, L, P, and E-selectins, etc.). These substances all act in concert to attract circulating leukocytes and facilitate adherence [178–182]. Vasoactive substances released from activated leukocytes, platelets, and endothelial cells include platelet-activating factor (PAF), thromboxane (TBX),

leukotrienes (LTE), nitric oxide (NO), histamine, bradykinin, and prostaglandins (PGE) [183, 184]. These substances are all necessary to attract immune cells (primarily PMNs) to the site of infection and to facilitate pathogen containment. Stimulated endothelium can be a double-edged sword, however, because excessive activation can lead to vascular dilation and leak which are a driving forces behind the severe consequences of septic shock [178, 185]. Systemic overproduction of cytokines and vasoactive substances including NO are associated with circulatory alterations and organ failure. The balance of NO and endothelin-1 (ET-1), which is a vasoconstrictor, may be disrupted with endothelial damage, favoring the constrictive effects of ET-1 leading to ischemia and injury [186]. This phenomenon may explain in part why NO inhibitors increased mortality in adults with septic shock [187]. Glucocorticoid receptor is the target for the endogenous, adrenally produced steroid corticosterone. Endothelial GR is a critical negative regulator of NO synthase expression and NF-κB activation [188], demonstrating its role of endothelium in protecting the host during sepsis. Recent studies revealed a potential role of plasma angiopoietin protein during pediatric septic shock [189]. Angiopoietin-1 (protects against vascular leak) was reduced while angiopoietin-2 (promotes vascular permeability) was elevated highlighting a novel potential therapeutic opportunity to reduce end organ injury with pediatric septic shock.

Activated or damaged endothelium establishes a prothrombotic environment that can result in local microvascular occlusion [154] or progress to DIC [190]. Endothelial cell apoptosis, detachment from the lamina, and alterations in vascular tone combine to promote capillary leak of proteins and fluid leading to hypovolemia and shock [191]. Using transgenic mice, it was recently shown that pulmonary endothelial cells sense bloodborne bacteria and their products [178] while alveolar macrophages patrol the airspaces for pathogens [192]. These data illustrate the role of endothelium help to explain in part the occurrence of ARDS and PPHN associated with severe sepsis in the absence of a primary pulmonary infectious focus.

Pathophysiology of Sepsis

Cardiovascular

The most common organ dysfunction associated with sepsis is cardiovascular. As discussed above, septic shock is really a composite of hypovolemic, cardiogenic, and distributive shock. Distributive shock is related to endothelial NO production that leads to excessive vasodilation. Cardiogenic shock may be related to mitochondrial death (induced by RNI and ROI) with subsequent myocardial dysfunction. In addition, some of the mediators released by the innate

immune system are likely myocardial depressant factors [193]. Hypovolemia (absolute or relative) is very common. Peripheral vasoregulation abnormalities and myocardial dysfunction may play a larger role in hemodynamic derangements in pediatric patients, especially infants and neonates. Factors contributing to developmental differences in hemodynamic responses include altered structure and function of cardiomyocytes, limited ability to increase stroke volume and contractility, and contributions of the transition from fetal to neonatal circulation [65, 194–197]. In adults, septic shock is most commonly characterized by reduced systemic vascular resistance and elevated cardiac index [198]. In children, as discussed above, reduced cardiac output and increased systemic vascular resistance is more common [57–64]. The hemodynamic presentation in neonates is much more variable [199] and complicated by an unclear association between a normal blood pressure and adequate systemic blood flow [200, 201]. Myocardial dysfunction can lead to ventricular wall stretch that in turn elevates brain natriuretic peptide (BNP). BNP levels have utility as prognostic indicators of mortality [202] in older patients with sepsis and in post-operative children [203].

Immune System

Following severe sepsis or septic shock, there is an increased risk of subsequent infection and mortality [204]. This phenomena is termed "immunoparalysis" and is associated with reduced MHC class 2 expression and TNF-α production by mononuclear cells following endotoxin stimulation. In addition to altered monocytic responses, there is significant loss of lymphoid CD4+ T and B cells via caspase-dependent apoptotic pathways [205, 206]. Whether by clonal selection, apoptosis, or elevated endogenous glucocorticoid levels [207–209], lymphocyte loss may lead to a state of immune compromise following the acute phase of sepsis [207, 209–214]. New data suggests that IL-7 may play an important role in promoting T-cell activation and the prevention of apoptosis [215]. The importance of immunoparalysis has been convincingly demonstrated in infected adults [216–219] and children [204]. However, immunoparalysis following sepsis may not impact the preterm neonate in whom adaptive immune function is less well developed [220, 221].

Mechanisms behind sepsis and post-sepsis immune alterations are beginning to emerge. The intensity of the inflammatory response may be modified by neural-based mechanisms. T cell-secreted acetylcholine acts on macrophages to reduce production of TNF, IL-1, IL-18, HMGB1, and other cytokines [222]. The role of vagal tone in the pediatric host response to sepsis is unclear. Discovery and characterization of the impact of epigenetic-mediated immune system functional alterations following sepsis is an area of

intense research. DNA methylation as well as post-translational modification of histone proteins (methylation, acetylation, phosphorylation, ubiquitination, sumoylation) may occur after sepsis [195, 223]. These DNA alterations may modify transcription factor access of gene-specific promoter regions ultimately leading to short and long-term changes in gene expression and immune function.

In adults, absence or dysfunction of the adaptive immune system has a profound impact on survival in preclinical models [205]. Genome-wide mRNA expression profiling (GWEP) during pediatric septic shock revealed widespread repression of gene pathways corresponding to the adaptive immune system [224]. In adult animals, B cells (and in particular B cell cytokine production) and not T cells were shown to be important in the early host response [225]. Interestingly, experimental data using neonatal mice lacking an adaptive immune system (RAG-1$^{-/-}$) showed no difference in early polymicrobial sepsis survival as compared to wild-type animals with a present adaptive immune system [226]. As these findings illustrate, the contribution of adaptive immunity for protection and response against sepsis, and in particular which components are protective, is unclear in the most immature and requires further investigation.

Pulmonary

Acute hypoxic respiratory failure, acute respiratory distress syndrome, and acute lung injury are common pulmonary complications associated with severe sepsis. Destruction of the alveolar capillary membrane leads to refractory hypoxemia. Following direct or indirect insults to the lung, alveolar macrophages produce chemokines that mitigate PMN influx to lung parenchyma. Activated PMNs release ROI/RNI that damage endothelial and epithelial barriers leading to leakage of protein-rich edema fluid into the air spaces. Other pulmonary complications with severe sepsis may include secondary surfactant deficiency [227], pulmonary edema, primary or secondary pneumonia [228], and reactive pulmonary hypertension [194, 229].

Renal

Infection is an important predictor of acute kidney injury in children [230]. Over 40 % of septic children or adults manifest AKI on the first day of hospitalization. The pathophysiology of AKI with sepsis is incompletely characterized but historically been attributed to ischemia-reperfusion injury following cardiogenic and hypovolemic shock. More recently, other factors including patient age, body-mass index, malignancy, intra-abdominal source of infection, microcirculatory dysfunction, kidney energy failure, immune-mediated injury and

apoptosis, and nephrotoxin exposure also contribute to the development of AKI with sepsis.

Hepatic

Hepatic injury and dysfunction are frequent associations with severe sepsis. Mechanisms include reduced hepatic perfusion associated with septic shock and mitochondrial energy failure. Reductions in coagulation and complement factors, acute phase reactants as well as increases in transaminases and bilirubin are commonly seen especially with decreased perfusion states. Cytokines and nitric oxide reduce CYP 450 activity in vitro and in vivo. CYP 450-mediated drug metabolism is decreased in children with sepsis, related in part to the degree of inflammation and organ failure [231]. Hepatocyte destruction and lymphocyte infiltration are associated with increased soluble Fas (CD95, sFas) and sFas ligand levels [232]. Plasma sFas levels are increased with severe sepsis, persistent multiorgan failure (MOF), mortality, and correlates with serum IL-6, IL-10, nitrite + nitrate levels. Plasma sFasL is increased in liver failure-associated MOF, mortality, and was associated with viral infection but was not increased in severe sepsis and did not correlate with inflammation.

Multi-organ Dysfunction Syndrome (MODS)

Sepsis that leads to multi-organ dysfunction syndrome (MODS) carries a bleak prognosis. Inadequate cardiac output and microcirculatory failure, which may be combined with formation of microthrombi and DIC, can lead to poor perfusion to the kidney [233, 234], liver [235], gut [236], and CNS [237–241]. Recent studies suggest that the mechanism of organ failure in sepsis may relate to decreased oxygen utilization associated with mitochondrial dysfunction rather than or in addition to poor oxygen delivery to tissues [242, 243]. Mitochondrial dysfunction can initiate activation of cell death pathways including apoptosis, pyroptosis, necrosis, and NETosis (i.e., cell death mediated by neutrophil extracelluar traps). DAMPs (including nucleosomes and microparticles) created by activation of these cell death programs further amplify the host immune response.

BPI is a component of PMN granules that is bactericidal towards gram-negative bacteria and inhibits LPS-mediated inflammatory responses. Plasma BPI concentrations in critically ill children with sepsis syndrome or organ system failure both had higher median plasma BPI concentrations than critically ill controls without sepsis or organ failure. Plasma BPI concentrations were also positively associated with pediatric risk of mortality score [140]. Free radicals play an important role in the inflammatory process of sepsis [244].

In a piglet neonatal sepsis model edaravone, a novel free radical scavenger, increased MAP and cardiac output, lowered heart rate, hydroperoxide, nitrite, and nitrate levels, delayed the TNF-alpha surge, prevented HMGB1 elevation, and was associated with longer survival times [245]. Elevated plasma nitrite/nitrates and increased organ failure scores are present in children with sepsis with an exaggerated proinflammatory state and a robust anti-inflammatory response [246]. Increased plasma nitrite and nitrate concentrations are associated with the development of multiple organ failure in pediatric sepsis [247].

Several other mediators of MODS have been recently described and are currently under investigation. Elevated MMP8 mRNA expression and activity in septic shock correlates with decreased survival and increased organ failure in pediatric patients. MMP8 is a direct activator of NF-κB [248]. Inhibition (genetic or pharmacologic) of MMP8 leads to improved survival and a blunted inflammatory profile in a murine model of sepsis. GWEP revealed zinc homeostasis as an important feature of pediatric sepsis [249–251]. Prophylactic zinc supplementation reduced bacterial load and mortality in a murine model of peritoneal sepsis [252]. PBMC PPAR-α gene expression is decreased with severe sepsis. PPAR-α knockout mice exhibit a decreased inflammatory response and poor bacterial clearance in septic animals [253]. PPAR-γ exhibits altered expression and activity in PBMCs from children with septic shock [254]. Kruppel-like transcription factor 2 (KLF2) is a potent regulator of myeloid cell activation. Myeloid cell KLF2 expression is reduced with hypoxia and exposure to bacterial products, and a reduction of KLF2 is associated with increased NF-κB mediated HIF-1α transcription [255].

Management of Sepsis

Over two decades of clinical research looking searching for the so-called "magic bullet," or a mediator-specific therapeutic agent designed to abrogate a specific target in the host inflammatory response to sepsis, have been largely disappointing [256]. Several experts have speculated on why the vast majority of therapeutic trials in sepsis have failed [183, 257–259]. One commonly cited reason is the sheer complexity of the sepsis phenotype. As discussed above, there are several hundred genes that are differentially expressed in sepsis [249, 251, 260–262], many of which are involved in redundant and overlapping pathways. Aside from prevention (which is of significant importance) then [4, 263], the crux of pediatric sepsis management rests upon three important therapeutic principles or pillars – (i) early recognition of sepsis, (ii) early source control and antibiotic administration, and (iii) early reversal of the shock state [6]. These three pillars are based upon the American College of Critical Care Medicine's consensus guidelines for the management of critically ill neonates and children with septic shock (Fig. 30.4) [67].

Early Recognition

Early recognition and treatment is the cornerstone of sepsis therapy and represents the first pillar in management. Unfortunately, early recognition and diagnosis of sepsis, especially in children, remains a significant challenge, even in developed countries with advanced health care delivery systems [4, 256, 264]. Failure to recognize the signs and symptoms of sepsis and to institute timely and appropriate care leads to higher mortality rates in children and adults [54, 265–271]. There are no simple, straightforward blood tests that can reliably diagnose patients with sepsis. While early evidence suggests that the biomarker procalcitonin is more sensitive than C-reactive protein (CRP) in detecting serious bacterial illness in children with fever [272], additional studies are required. The currently available test for procalcitonin is relatively inexpensive and readily available. Interleukin (IL)-27 may also be a more accurate biomarker compared to CRP, especially when used in conjunction with procalcitonin – though again further studies are required [273, 274]. Until these biomarkers are studied more extensively, sepsis will remain primarily a clinical diagnosis based upon the recognition of a constellation of signs and symptoms (i.e., SIRS) that occur with infection.

As Machiavelli stated in the quote at the beginning of this chapter, in its early stages, when treatment is most effective, sepsis is virtually indistinguishable from other, more benign febrile illnesses. Early recognition of sepsis therefore requires a heightened awareness and increased index of suspicion on the part of the clinician, and traditionally a substantial amount of clinical experience [275–277]. Sustained tachycardia in absence of fever, tachypnea or respiratory distress in absence of pulmonary disease or out of proportion to other symptoms, subtle changes in skin perfusion, and altered mental status are all highly suggestive of possible sepsis.

Early Source Control and Antibiotic Administration

Early source control and antibiotic administration represents the second pillar in the successful management of pediatric sepsis. Autopsy studies in adults have shown that the failure to diagnose and appropriately treat infections with antibiotics or surgical drainage is the most common avoidable error in the treatment of sepsis [278, 279]. Multiple studies have demonstrated the importance of early source control (which may include surgical removal of the nidus of infection) and

Fig. 30.4 American College of Medicine consensus guidelines for the management of critically ill children with septic shock (Reprinted from Brierley et al. [67]. With permission from Wolters Kluwer Health)
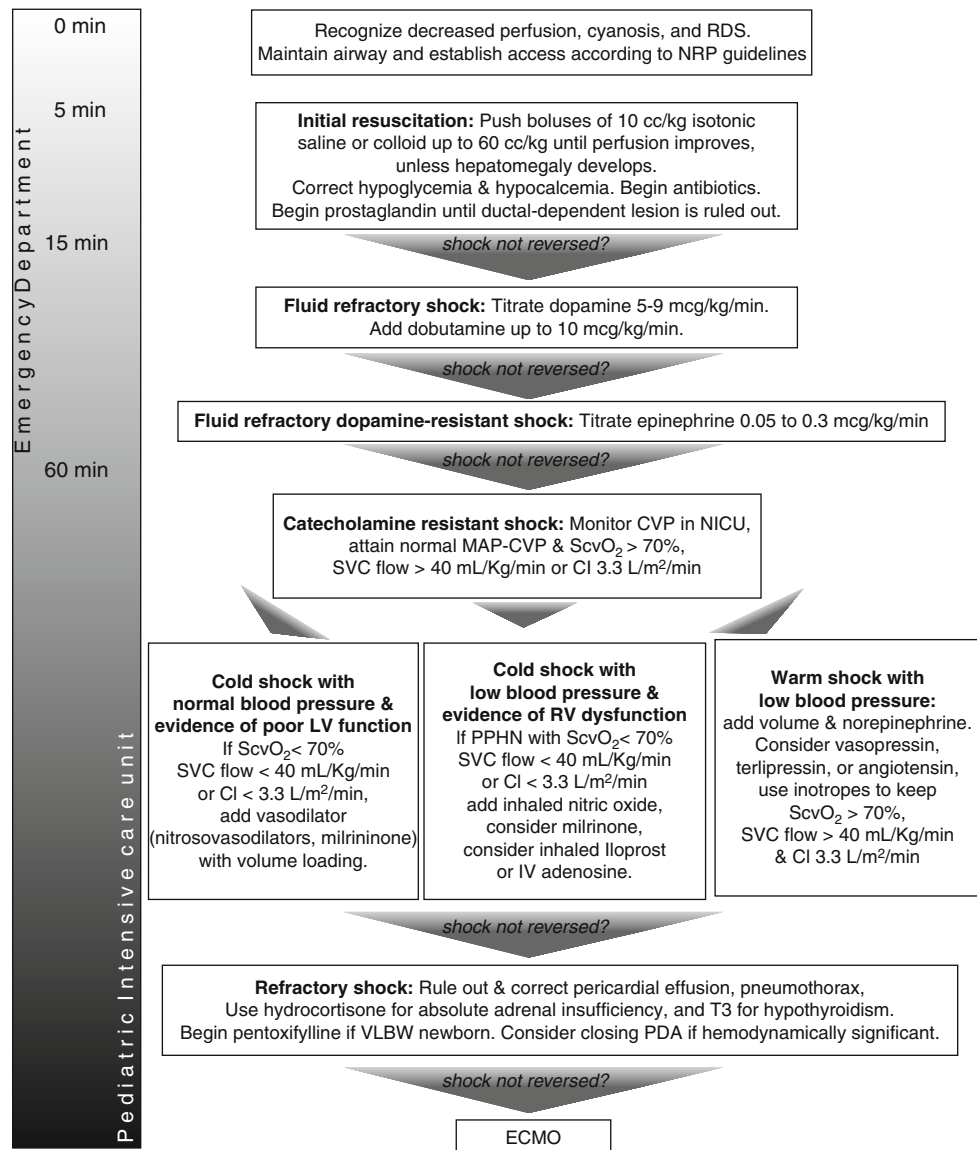
antibiotic administration. For example, Gaieski and colleagues showed that mortality significantly increased when antibiotics were delayed beyond 1 h in critically ill adults presenting to the ED with severe sepsis and septic shock [280]. A study by Kumar and colleagues showed that only 50 % of patients received antibiotics within 6 h of documented hypotension, which was associated with increased risk for mortality [267]. A protocolized approach to the management of sepsis has been shown to improve the time to administration of antibiotics in several studies involving both children [281–283] and adults presenting to the ED [284–289]. Indeed, this kind of approach has even been successful in resource-poor countries. For example, studies conducted in a rural population in India showed that home administration of oral and injectable antibiotics to neonates with perinatal-acquired sepsis resulted in significant reductions in

sepsis-related neonatal mortality – sustained over a 10-year period [290, 291].

Early source control and antibiotic administration have also been emphasized in the highly successful Surviving Sepsis Campaign (SSC) [292], a joint collaboration between the European Society of Intensive Care Medicine, the International Sepsis Forum, and the Society of Critical Care Medicine. The resuscitation bundle for managing critically ill adults with sepsis calls for broad-spectrum antibiotics to be administered within 3 h of ED admission and/or 1 h of ICU admission [293–295]. Notably, the SSC (discussed further below) enrolled over 15,022 patients from 165 centers from January, 2005 through March, 2008. Compliance with the resuscitation and management bundles was associated with a significant reduction in hospital mortality [296].

Early Reversal of Shock

Early reversal of the shock state with aggressive resuscitation targeted at rational therapeutic endpoints represents the third pillar in the management of pediatric sepsis. While it has been known for several years that early reversal of shock improves outcomes in critically ill children [54, 269, 297], the concept known as *early goal-directed therapy* (EGDT) was initially popularized following the publication of the study by Rivers and colleagues, which showed that EGDT significantly improved outcomes [298]. All patients had arterial and central venous catheters placed in the ED, and therapy was protocolized to achieve a CVP 8–12 mmHg with fluid resuscitation, MAP >65 mmHg and <95 mmHg with vasoactive agents, and a superior vena caval oxygen saturation ($S_{svc}O_2$) greater than 70 % with inotropes and blood transfusion. Importantly, treatment in the conventional therapy group was targeted towards a CVP 8–12 mmHg, MAP >65 mmHg and <95 mmHg, and urine output >5 mL/kg/h. In-hospital mortality was significantly lower in the EGDT group compared to the conventional therapy group (30.5 % vs. 46.5 %, respectively, p=0.009). Differences in outcomes were noted despite the fact that the groups received the same treatment after the initial 6 h of therapy.

The EGDT trial is one of only a few studies that have been published to date that have shown a reduction in mortality from sepsis and highlights the importance of early recognition and aggressive resuscitation of shock. A subsequent meta-analysis of 21 randomized, controlled trials of sepsis by Kern and Shoemaker [299] further highlights the importance of early, goal-directed therapy, demonstrating that when patients with acute critical illness are treated early to achieve optimal goals before the development of organ failure, significant reductions in mortality are achieved [294, 300]. In addition, there are currently three prospective, multicenter, randomized clinical trials going on in the United Kingdom, U.S., and Australia that are further testing the overall validity of this concept in critically ill adults with severe sepsis/septic shock. The Surviving Sepsis Campaign conducted a multicenter, international, prospective, collaborative involving over 15,000 patients worldwide. Increasing compliance with a 6-h resuscitation bundle (focused on early antibiotics and rapid reversal of shock) and maintenance bundle (focused more on lung-protective ventilation, stress ulcer prophylaxis, glucose control, corticosteroids for vasopressor-refractory shock, activated protein C) was associated with significant reductions in mortality at participating hospitals [296]. There has since been significant controversy regarding both the use of activated Protein C and "tight glucose control" in critically ill patients with severe sepsis and septic shock. A similar quasi-experimental study in Spain showed that compliance with the resuscitation bundle was more important than the maintenance bundle [301].

Table 30.5 Surviving Sepsis Campaign (*SSC*) bundles

To be completed within 3 h
1. Measure lactate level
2. Obtain blood cultures prior to administration of antibiotics
3. Administer broad spectrum antibiotics
4. Administer 30 mL/kg crystalloid for hypotension or lactate ≥4 mmol/L

To be complete within 6 h
1. Apply vasopressors (for fluid-refractory hypotension) to maintain a mean arterial blood pressure ≥65 mmHg (adult)
2. If the patient remains hypotensive despite fluid resuscitation or initial lactate ≥4 mmo/L
(a) Measure central venous pressure (*CVP*)
(b) Measure central venous oxygen saturation ($S_{Cv}O_2$)
Remeasure lactate if initial lactate was elevated

Based on these results and the aforementioned controversies, the Surviving Sepsis Campaign has changed the bundles to de-emphasize care beyond 6 h (see the Surviving Sepsis Campaign website at http://www.survivingsepsis.org/Bundles/Pages/default.aspx) (Table 30.5).

Preliminary studies suggest that EGDT may be beneficial in select groups of critically ill children with sepsis [54, 282, 283, 302, 303]. Additionally, investigators at St. Mary's Hospital-London developed a protocol for managing critically ill children with meningococcal sepsis [297] and were able to demonstrate a dramatic reduction in mortality from 23 % in 1993 to 2 % in 1997 [266]. This protocol similarly emphasized early recognition and has been modified [269] and adapted into a National Institute for Health and Clinical Excellence (NICE) guideline in the United Kingdom [304].

Based upon the studies published above, it is imperative that resuscitation should begin as soon as the sepsis syndrome is recognized, usually in the ED, and should not be delayed until admission to the PICU [54, 57, 305, 306]. During the first 6 h the aim is to achieve the following goals:

1. Capillary refill <2 s
2. Urine output ≥1.0 mL/kg/h
3. Normal pulses, without difference between peripheral and central pulse
4. A central venous oxygen saturation ($S_{svc}O_2$) of >70 %
5. Declining lactate and base deficit
6. Improved level of consciousness

Fluid Resuscitation

Critically ill children with severe sepsis and/or septic shock almost universally have decreased effective intravascular volume for a variety of reasons. Many of these children have had poor oral intake of fluid for a period of time prior to developing sepsis. With the development of increased vascular permeability, intravascular volume has been lost due to third spacing. Finally, peripheral vasodilation related to excessive NO production (see above) results in an abnormally

increased vascular capacitance, decreasing the effective circulating volume. Aggressive fluid resuscitation with crystalloids or colloids is therefore essential to increase survival of septic shock in children. This goal can be reached by administering 20 mL/kg boluses over 5-10 min, repeated as necessary to achieve the hemodynamic goals stated in the preceding paragraph [305, 306]. Large fluid deficits are typically encountered, requiring 60 mL/kg volume resuscitation or occasionally much higher amounts [305]. Transfusion of packed red blood cells should be considered to maintain hemoglobin levels at levels consistent with adequate oxygen delivery, though the optimal hemoglobin for infants and children with septic shock is not currently known.

The available evidence suggests that the different types of intravenous fluid available for fluid resuscitation are similar in terms of efficacy, both in pediatric [307–310] and adult studies [311–313]. There is perhaps one caveat, in that there is some preliminary data suggesting a mortality benefit with colloid compared to crystalloid in critically ill children with severe sepsis secondary to malaria [314, 315]. In recent years, several studies have shown a correlation between fluid overload and increased mortality in critically ill children requiring renal replacement therapy [316–319]. Fluid overload has also been shown to correlate with impaired oxygenation, longer duration of mechanical ventilation, and increased PICU and hospital length of stay (LOS), even in critically ill children who do not require renal replacement therapy [320]. Preliminary data from our group suggests that fluid overload is associated with worse outcomes in critically ill children with a low initial probability of mortality, but not in children with an intermediate-risk or high-risk of mortality (*Wong, personal communication*).

The recently published FEAST trial has led to further questions on the utility of fluid resuscitation in critically ill children with severe sepsis and septic shock. In this prospective, randomized, controlled study, children presenting to the hospital (in Uganda, Kenya, or Tanzania) with a severe febrile illness received one of three treatments: 20–40 mL/kg 5 % albumin, 20–40 mL/kg 0.9 % saline, or no fluid bolus. There were no differences in mortality at 48 h between the saline (110/1,047 children, 10.5 %) and albumin (111/1,050 children, 10.6 %) groups, though mortality was lowest (76/1,044 children, 7.3 %) in the control group that did not receive a fluid bolus. These results were consistent across all sub-group analyses [321]. A follow-up analysis of these results showed that excess mortality occurred as a result of cardiovascular collapse and not fluid overload (pulmonary edema, neurologic deterioration, etc.) [322]. Given the accumulation of several decades of experience with intravenous fluid resuscitation, it would seem premature to abandon (or even temper) this therapy without additional data. In addition, it should be noted that the FEAST study and the majority of the aforementioned studies comparing crystalloid to colloid in children were performed in patient populations (e.g., dengue fever, severe malaria, severe malnutrition,

etc.) that are quite different from the majority of critically ill children with shock in the developed world. Whether these findings can be generalized to different populations around the world remains an area of active study. In light of the remaining questions and available data, the usual practice in most PICUs is to initiate volume resuscitation with crystalloid fluids as a first line and follow with colloid if needed.

Collectively, these data further strengthen the concept of carefully titrating fluid resuscitation and other therapies to rational therapeutic endpoints in a protocolized fashion, consistent with the American College of Critical Care Medicine Clinical Guidelines for Hemodynamic Support of Neonates and Children with Septic Shock [67]. Again, reliable implementation of these guidelines has resulted in improved outcomes in at least a few published reports [54, 302, 303].

Hemodynamic Support

While the principles of hemodynamic monitoring and support have been discussed elsewhere in this textbook, a brief discussion here is justified. In the case of fluid refractory shock, inotropic or vasoactive support should be started without delay. Again, the most common hemodynamic derangements observed in critically ill children with septic shock are low cardiac output and high systemic vascular resistance (i.e., so-called *cold shock*). Dopamine has traditionally been the initial agent of choice for infants and children with septic shock [306], though the choice of additional agents will be determined by the clinical condition of the child. For example, dobutamine or epinephrine may be preferable in children with low cardiac output and poor peripheral perfusion (*cold shock*), especially given the association of dopamine with increased morbidity and mortality in several studies [323]. In addition, there have been some observational studies in both critically ill children and adults suggesting that dopamine may be associated with an increased risk of morbidity and mortality [324–326]. Norepinephrine is the preferred agent for children with normal cardiac output and bounding peripheral pulses (*warm shock*). A type III phosphodiesterase inhibitor such as milrinone may also improve perfusion in children with *cold shock*, who are adequately volume resuscitated, though hypotension is always a risk with this agent. Regardless of the vasoactive regimen chosen, close monitoring of the oxygen delivery, as assessed by end-organ function, mixed venous oxygen saturation and a falling lactate levels, is necessary to tailor ongoing treatment. Finally, extracorporeal life support (ECLS) may be necessary and lifesaving in select patients [327–332].

Supportive Care

Children with sepsis may have poor nutrition prior to presentation and are often not fed in the first few days of illness. Because of increased metabolic rate and poor nutrition,

septic patients are frequently catabolic and at risk for the development of protein calorie malnutrition [333]. Intestinal ischemia in association with the loss of the mucosal barrier from malnutrition has been associated with translocation of bacteria and endotoxin from the intestine into the blood stream [334, 335]. Use of enteral feeding in critical illness has been shown to improve survival and decrease hospital stay [336]. The benefit of enteral feeding should be balanced with the risk of stressing the intestinal function in the face of poor splanchnic perfusion, especially in the presence of vasopressors such as epinephrine and norepinephrine [337, 338]. Regardless of the mode of feeding, adequate nutrition and nitrogen balance is important for maintaining adequate host immune function and achieving homeostasis. Malnutrition may adversely affect the immune function and the ability to have an appropriate immune response [339]. Finally, in the absence of enteral feedings, protection from stress-related gastrointestinal bleeding is advised.

Corticosteroids

High pharmacologic doses of corticosteroids were used in the past in an attempt to turn off the systemic inflammatory response associated with sepsis. However, most of the studies suggested that high-dose corticosteroids did not improve survival in septic patients and may even have worsened outcome by increasing the incidence of secondary infection [340]. However, more recent data suggests that patients, who are critically ill, in persistent shock, requiring vasopressors and mechanical ventilation, might benefit from physiological doses of corticosteroids [341]. This finding has not been confirmed in other studies [342]. Some investigators have postulated that some patients with sepsis may have a relative adrenal insufficiency despite normal serum cortisol levels, because of desensitization of corticosteroid responsiveness [343, 344]. This has been shown in critically ill children with meningococcal sepsis [345] and in other forms of sepsis [343]. Unfortunately, there have been no randomized, controlled studies of corticosteroid replacement in critically ill children with severe sepsis or septic shock. The most recent Surviving Sepsis Campaign guidelines [300] recommend corticosteroids only if the patient remains refractory to vasoactive medications. In addition, use of the ACTH stimulation test to discern between relative and absolute adrenal insufficiency is not necessary. The pediatric version of these guidelines suggests starting stress-dose hydrocortisone (50–100 mg/m^2/day hydrocortisone) in critically ill children with fluid-refractory and catecholamine-resistant septic shock and suspected or proven absolute adrenal insufficiency (i.e., after appropriate diagnostic workup with a random/basal serum cortisol level followed by an ACTH stimulation test). The diagnosis and treatment of adrenal insufficiency is discussed in great detail in other chapters of this textbook.

Conclusion

Sepsis remains a significant problem in pediatrics. The diagnosis remains a clinical one, and early recognition is absolutely critical. Additional treatment depends upon early source control and antibiotic administration, as well as early reversal of shock.

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