Journal of Medical Sciences (2010); 3(1): 11-27

Review Article

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Neonatal Sepsis in the Very Low Birth Weight Preterm Infants: Part 2: Review of Definition, Diagnosis and Management

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Abstract

Background: Having presented brief epidemiology of neonatal infection and patho-physiology of neonatal sepsis in the first part of this review we now address the difficulties in defining, diagnosing and treating neonatal sepsis.

Objective: The objective of this part of the review is firstly, to highlight the reasons for lack of consensus on the definition of neonatal sepsis despite a number of international conferences of experts on the subject. Secondly, to discuss the increasing sophistication of available laboratory tests and why they all lack the certainty desired by the clinician and thirdly to discuss the various evidence based treatment modalities available to treat neonatal sepsis.

Conclusion: It is suggested that pragmatic definition of sepsis as suggested by us should be adopted. Greater use of biomarkers and molecular tests should be made to diagnose sepsis early and accurately. Lastly, it is hoped to change the clinician's paradigm by using evidence based management care bundle/package that includes adjunctive immune-modulatory and host defence boosting drugs.

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Keywords: ***Neonatal sepsis, patho-physiology, diagnosis, management, very low birth weight infant (VLBW).***

Introduction

Webster English dictionary[1] describes sepsis as "putrefaction", i.e. decomposition of organic matter (by bacteria or fungi) resulting from interaction between germ and host. Joseph Carcillo[2] has suggested that 'sepsis' is when systemic inflammatory response syndrome (SIRS) occurs in the presence of a "living infection". Despite numerous consensus conferences there is still no agreed definition of sepsis! The reasons for this are complex, reflecting the marked clinical and biochemical heterogeneity observed in the affected septic individuals due to their genetic variation, environment, state of hosts defence system and characteristics of the pathogen/s involved. Similarly, till very recently there has been no clear definition of blood stream infection in the neonatal period[3,4] and there is still no consensus as to what constitutes sepsis or septic shock in the newborn, though akin to adults it is not an infrequent problem. Lack of consensus also highlights the fact that sepsis far from being a homogenous condition reflects a continuum from foetal inflammatory response syndrome (FIRS) *{akin to systemic inflammatory response syndrome (SIRS) described in adults}* to sepsis, severe sepsis, septic shock, multi-organ failure and death (*Fig. 1*). The infected infant moving from one phase to another in either direction imperceptibly.[5] In a study of 908 out of 1612 infants admitted to our neonatal intensive care unit between 1st January 1996 and 31st December 2000 who were suspected and investigated for sepsis; using regression analysis

we[6] found that the most significant clinical findings for sepsis were presence of tachyapnoea with grunting/chest retraction or apnoea, temperature instability and a capillary refill time of greater than 3 seconds. Of the laboratory tests leucopoenia (<4000 X 10^9 /L) or leucocytosis (>34,000 X 10^9 /L), C-reactive protein greater than 10 mg/dl and interleukin 8 value of greater than 70 pg/ml were the most important variables. Based on these findings, evidence from literature, and an international consensus conference of experts in 2004 we have suggested a pragmatic and user friendly definition of neonatal sepsis[3] in which we define sepsis as the presence of two or more of clinical features plus one or more of laboratory parameters outlined below with or without positive blood culture;

- Presence of Tachypnoea (respiratory rate > 60 bpm) **plus** grunting/retraction or desaturation.
- Temperature instability (< 36 ^{0} C or > 37.9 ^{0} C)
- Capillary Refill time > 3 seconds
- White Blood Cell count (< 4000 X 10^9 /L or > 34,000 X 10^9 /L)
- C-Reactive Protein > 10 mg/dl
- IL-6 or IL-8 > 70 pg/ml
- 16SrRNA gene PCR: Positive

Diagnosis

Fischer[7] has very elegantly demonstrated that though the ability of a senior experienced clinician to diagnose sepsis is high, there is still lack of diagnostic certainty at the cot side which is often influenced by the presence or absence of 'risk factors', (described in Part 1 of this review), lack of specific clinical signs and symptoms, differing patho-physiology and crucially the lack of highly sensitive and specific laboratory test.

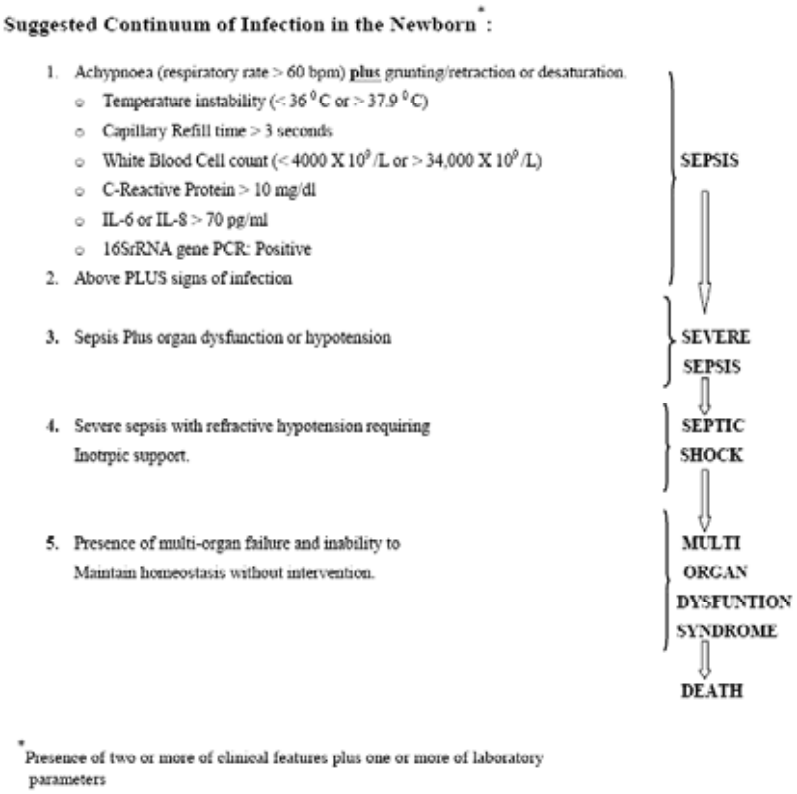

Fig. (1). Suggested continuum of sepsis.

Table 1. Most frequent signs and symptoms in neonatal sepsis

Symptoms	Signs
Lethargy	Temperature instability
Poor feeding	Prolonged capillary filling time
	'Does not look well'.
Apnea	Widening toe–core temperature difference, Variability in heart rate
Respiratory distress	Hepato/splenomegaly
	Pallor. Unexplained anaemia/Thrombocytopenia
	Mottling
Cyanosis	Abnormal neurological reflexes
Abdominal distention	Glucose intolerance: Hyper/hypoglycemia
Vomiting/increasing gastric	Glucosuria
	Residue
Jaundice	Persistent acidosis
	Petechiae, purpura, bleeding
Irritability/Seizures	
Sclerema	

Of the known 'risk factors' enumerated earlier we have reported that the two most important risk factors are; birth at or before 31 weeks of gestation (OR 3.9, 95% CI 1.4-11.0) and or birth weight less than 1500 Grams (OR 5.7, 95% CI 2.5-15.6).[6]

Clinical signs and symptoms (*Table 1*) of neonatal sepsis are non-specific because they are often associated with characteristics of the causative organism and the body's response to the pathogen/s. These non-specific signs and symptoms are also either common to or associated with other neonatal conditions like respiratory distress syndrome, metabolic disorders, and intracranial bleeding. Signs and symptoms like temperature instability, changes in heart rate or its variability,[8] apnoea, prolonged capillary refill time, hypotension and or decreased urine output, persistent metabolic acidosis, hypo or hyperglycaemia individually have low sensitivity and specificity with none exceeding the likelihood ratio of 15%.[9,10] Added to this, are the ever changing metabolic changes due to sepsis that are reflected in the constantly changing signs and symptoms in sepsis. These changes vary from initial phase of hypo-metabolism (*temperature and heart rate variability*), decreased energy expenditure *(lethargy)*, decreased cardiac output *(hypotension, prolonged capillary refill time)*, lower oxygen consumption and vasoconstriction *(peripheral cyanosis, apnoea)* to the later phase of hyper-metabolism, increased energy expenditure *(irritability, increased oxygen requirement)*, increased cardiac output *(tachycardia)* and high oxygen consumption *(cyanotic episodes)*.[11]

Diagnostic Tests

Clinicians in search of certainty of diagnosis have long sought biological marker/s that would provide them with early and accurate diagnosis or markers that would also provide guidance to treatment.[12,13] There is an increasing array of laboratory tests for diagnosis of sepsis but despite initial enthusiasm most have failed to reach the level of accuracy and consistency or practical utility required by

the practicing clinician. For the most commonly used diagnostic test (*Full blood count, Blood culture, CRP*) the chances that infection is present are less than 50% when taken on their own. Thus, for a greater degree of certainty clinicians frequently use combination of biological markers to improve their predictive ability. Some newer test, or combinations however provide high positive (PPV) and negative (NPV) predictive values but they are either expensive or not universally available and where they are, clinicians need a paradigm change to use them more frequently. Below we discuss most frequently used current diagnostic methodologies, their advantages and disadvantages and suggest the potential advantages of using tests that measure immune response of the patient to diagnose and monitor sepsis.

Blood culture remains the 'gold standard' but is often unreliable when intra-partum anti-biotics have been administered to the mother. Blood culture also fails to detect bacteraemia in 27%-92% of preterm VLBW infants.[14] This is often due to the volume of blood inoculated into the blood culture bottle being insufficient[15] or suboptimal processing of the specimen, but perhaps the most important reason is that bacteremia is often transitory or intermittent. Yield from blood culture can be improved not by sending repeated small volume samples[16] but by inoculating a minimum of 0.5-1 ml of blood into the blood culture bottle[15]. Further difficulty with blood culture is its 'turn around' time of at least 18-24 hours; this is too long for a test on which clinical decisions have to be made. Recent automated culturing systems based on presence of CO_2, or pH provide higher degree of accuracy and a 'turn around' time between 12 and 36 hours.[15]

Biomarkers

There is an on going search for an ideal test or a biomarker that is accurate with high degree of sensitivity, specificity, PPV and NPV that could be delivered in real time. No such test has yet been described. The desire to find a single biomarker is fundamentally flawed and is unlikely to be fruitful because of the complexity and heterogeneity of the sepsis process described above. Moreover, it should be remembered that some tests no matter how sensitive are often negative when taken immediately at birth or before the onset of an inflammatory response. Never the less continued search to find improved methods for diagnosing and monitoring sepsis is exceedingly important when one considers the material and other cost of inappropriate use of antibiotics, drug resistance, increased length of hospital stay due to the uncertainty of clinical diagnosis.

Leukocyte number, character and indices are most frequently utilised to diagnose or monitor sepsis. In nearly 50% of infants with infection their values may be normal at the initial phase of infection only to become abnormal after 12 hours or so. Total leukocyte counts below 4000 x 10^9 /l or above 30,000 x 10^9 /l is considered abnormal with sensitivity between 17%-90%, and specificity 31%-100% .[17] Total immature neutrophils count of greater than 1%, or immature to total neutrophils ratio of greater than 0.02 has a PPV of only 23% but a NPV of 92%. If platelet count of less than 100,000/cu.mm is added to immature to total neutrophil ratio greater than 0.02 then the PPV increases to 43% and NPV to 96%[5,17] an important consideration in resource limited conditions.

Acute Phase Proteins: These are endogenous peptides produced mainly by the liver as a response to tissue injury, or infection. The most frequently used and most studied is CRP.

C-Reactive Protein (CRP). CRP is synthesized by the liver following IL-6 activation; it is involved in coagulation and opsonisation. CRP increases late in infection, with a lag time of 12-24 hours explaining the low sensitivity (60%) early in sepsis that increases to 84% by 48 hours after the onset of sepsis. Specificity and NPV also improve with time reaching 99%-100% by 48 hours of onset of infection.[18,19] It must be

remembered that neonate's capacity to produce CRP is lower than that of an adult another reason for its reduced sensitivity.[20] We[21] and others[17-22] have found serial measurements of CRP more helpful in determining duration of antibiotic therapy rather than its ability to diagnose sepsis.

Procalcitonin (PCT): PCT a 14-kDa protein that rises within 4 hours following onset of infection with a half life between 22 and 29 hours sometimes longer in sepsis.[23] It is produced by monocytes and the liver. Diagnostic utility of PCT in early onset sepsis is limited due to endogenous postnatal surge of PCT after birth peaking at 24 hours of postnatal age. PCT also has low sensitivity (81.4%), specificity (80.6%) and low NPV of 72% in premature infants.[23-25] Stocker et al have suggested serial PCT determinations allow shortening the duration of antibiotic therapy in term and near-term infants with suspected early-onset sepsis. Before routine PCT assessment or PCT-guided antibiotic strategy can be recommended, its usefulness has to be confirmed in a larger cohort of premature neonates.[26]

Inter-alpha Inhibitor Protein {Ialp}: This acute phase protein belongs to the family of serine protease inhibitors that are synthesized in the liver. Unlike other acute phase proteins Ialp is down regulated by inflammation. A recent study[27] in nineteen neonates has demonstrated decreased levels of Ialp in infants with sepsis. The numbers studied were small and the values overlapped with those in non-infected infants thus the accuracy of this test needs to be studied further.

Serum Amyloid A {SAA}: An acute phase protein induced by IL-1, TNF-α and IL-6. It increases by 8-24 hours after onset of infection and has a sensitivity of 96% with a NPV of 99%.[28] Though more robust than many other acute phase reactants larger studies are required to establish this as a routine test in neonates.

Other Acute Phase Proteins: There are a large number that have been studied e.g. neoptin, Lactoferrin, alpha-1-anti-trypsin, anti-thrombin, and others but none have gained popularity due to their poor sensitivity, specificity or technical problems.[13,29]

Cytokines and Chemokines: Recently there has been a flurry of interest in the possibility of using cytokines and chemokines to diagnose and monitor sepsis both in adults and in neonates. Initial measurements of pro-inflammatory cytokines like TNF-α, IL-2 and Interferon gamma (INFγ) were disappointing due to their very short half life (∞17minutes) leading to high false negative results. Measurements of circulating pro-inflammatory cytokines with longer half life have proven to be more fruitful. Levels above 70 pg/ml of IL-6 or IL-8 have a sensitivity of 77%-97%, specificity between 76%-93%, a PPV of 42% and NPV of 99%[5,29] in sepsis. Kauster *et al.*[30] noted that IL-6 actually increased two days prior to clinical diagnosis of sepsis in neonates suggesting that they may be very early markers of sepsis. Chemo-attractant IL-8 with a sensitivity of 92% and specificity of >70%, NPV of 94% appears to be a better marker of neonatal sepsis than IL-6.[5] Current interest is focused on IL-10 an anti-inflammatory cytokine which strongly inhibits pro-inflammatory cytokines like TNFα, interleukin 1, 6, 12 and 18 in additions to inhibiting translocation of nuclear factor-κB (NF-κB).[31] Anti-inflammatory cytokine IL-10 in combination with IL-6 and RANTES (regulated on activation normal T cell expressed and secreted) have recently been shown to diagnose disseminated intra-vascular coagulation secondary to sepsis with near absolute certainty (sensitivity 100%, specificity 97%, and NPV of 100%).[32]

Availability of semi-quantitative cot side measurement of IL-6 requiring only 50µl blood and a 'turn around' time of 20 minutes has brought the prospect of early cot side diagnosis a little closer but this method warrants robust clinical trial before it can be recommended. We (unpublished) using multiplex bead technology have studied an array of cytokines and chemokines in preterm

neonates with suspected and proven bacterial sepsis. With a drop of blood (<50 µl) collected on a Guthrie card and a two hour turn around time, we have evaluated 20 pro-inflammatory cyto and chemokines. In this pilot study of 60 infants with culture proven sepsis we found macrophage inflammatory protein (MIP-1ß) to be the most useful diagnostic and prognostic marker with sensitivity of 93% and specificity of 87% and a NPV of 98%. This needs to be evaluated further with a larger cohort of infants.

Molecular Markers: There has been a tremendous advance in the use of molecular techniques for diagnosis and monitoring sepsis. These tests are fast and reliable.

Polymerase Chain Reaction (PCR): PCR for bacterial 16SrRNA gene gives a sensitivity of 96%, specificity of 99.4%, PPV of 88.9% and NPV of 99.8%.[33] Using microarray hybridization technique PCR not only detects bacteremia but can also identify the offending organism.[34] Thus PCR has significant advantages over blood culture in that it has much higher accuracy, a short (4-6 hours) 'turn around' time and requires only 0.2-0.3 mls of blood but it is expensive and is not universally available. Commercial companies are developing machines to do PCR within neonatal units.

Soluble Triggering Receptor Expressed on Myeloid Cells (sTREM-1): This cytokine promoter has been evaluated as a potential marker for sepsis with sensitivity between 96%-98% and specificity 89%-90% in adults with pneumonia and other conditions associated with infection[35] We are currently evaluating sTREM-1 in neonates with suspected sepsis.

Cell Surface Antigens: Availability of flow cytometric analysis of cell surface markers has enabled the study of cell surface antigens in sepsis.[36] CD64 has sensitivity between 81%-96% and a NPV between 89%-97%.[37] Whilst promising, estimation of cell surface markers is limited by the need to process blood samples

*Table 2. Sensitivity and specificity of various laboratory tests for early diagnosis of neonatal sepsis**

Test	Sensitivity %	Specificity %	PPV	NPV
Individual Tests:				
Blood Culture	11 – 38	68 – 100	90 – 100	72 - 100
WBC < 4000 or > 30,000/cumm	17 – 90	31 – 100	50 – 86	60 - 89
I/T Ratio > 0.2	78	45	23	92
CRP > 2 mg/dl (EOS)	88	90	99	96
CRP > 2 mg/dl (LOS)	37	86	67	84
PCT > 2 ng/ml	92	97	45	50
IL-8 > 70 pg/ml	91	74	42	94
PCR. 16SrRNA	96	99	89	99
sTREM-1 > 60 ng/ml	96	89	86	96
CD 64	79	71	80	89
Combination Tests:				
I/T ratio + CRP	89	41	76	94
PCT +CRP	93	68	84	80
IL-8 + CRP	91	90	89	96

WBC: Total white blood count, I/T Ratio: Immature/Total neutrophils count, CRP: C-reactive protein
PCT: procalcitonin, sTREM-1: soluble trigger receptor expressed on myeloid cell, IL-8: Interleukin 8
*Adapted from various sources referenced in the text (mean values).

rapidly before neutrophils die from apoptosis or the surface antigens are down regulated plus the need for sophisticated equipment.

To summarise, diagnostic tests on the whole except perhaps PCR have poor or indeterminate accuracy and or are often not universally available.[38] None achieve the desired objectives of being quick, sensitive, and specific with high PPV and NPV. Unfortunately PCR is not commonly available 24 hours of the day in many institutions. Clinicians are therefore obliged to take a pragmatic view on how to use the commonly available tests either individually or as most do, use a combination to assist them in diagnosing and monitoring sepsis. A list of useful tests is given in *Table 2*.

Urine Examination: Due to difficulties with collection of clean samples and the risks associated with catheterisation or supra-pubic aspiration this important investigation is often not done. Low rate of urinary tract infection in the newborn has lead most authors to recommend against routine urine culture to diagnose sepsis.[39,40] There are inadequate studies evaluating true value of examining and culturing urine as part of every 'sepsis work-up'. Urine bacterial antigens are no substitutes as their accuracy is poor.[41]

Surface cultures (Skin, Ear, and Umbilicus) are not very helpful, their reliability is very poor and their routine use should be abandoned.[42]

Cerebrospinal Fluid (CSF)

There is considerable difference of opinion amongst clinicians and in literature whether CSF should be examined every time a 'sepsis work-up' is performed. Due to low rate of meningitis (1% of over 9000 blood culture positive infants[43]) many authors do not recommend routine lumbar puncture in the absence of a positive blood culture or localizing findings.[44,45] Current opinion varies from including CSF examination in every 'work-up', to examining the CSF when there are clinical features of meningitis or examining the CSF only when there is a positive blood culture. Data however suggests that as many as 38% of CSF culture-positive meningitis in neonates have negative blood culture taken at the same time!.[44] This may have to do with the problems associated with blood culture as enumerated earlier rather than true dichotomy between blood and CSF culture positivity rates. Never the less it remains a fact that meningitis can only be diagnosed or excluded if CSF is examined! We routinely include CSF examination in late onset sepsis evaluation but are selective in doing a lumbar puncture for early onset sepsis, a practice based on on-going surveillance data collected in our unit over last twenty years.

Management

Main objective of managing neonatal sepsis is to prevent it by reducing the source of bacterial entry into the neonate. This is best done by observing good hand hygiene, infection control techniques, avoiding unnecessary breaking of skin, using proper asepsis when skin has to be broken and intra-partum prophylaxis for maternal GBS carriage or PROM. There is some evidence that application of oil on the skin of VLBW infants reduces the rate of infection in these babies.[46]

Once the pathogen has entered the body then the aim of treatment is to kill the offending pathogen/s as quickly as possible, provide initial resuscitation if required, reduce/neutralise/eliminate bacterial toxins, regain the disturbed immunological and coagulation imbalance, boost host defences and most importantly correct the 'CHAOS' caused by the sepsis process itself (*Fig. 2*). This is a tall order and each element is as important as another and none can or should be ignored.

Thus, in an ideal world therapy for managing/treating neonatal sepsis should have the ability to kill the pathogen/s, increase macrophage surveillance, neutralise bacterial toxins, increase the number and function of neutrophils, improve opsonisation, phagocytosis and chemotaxis in addition to activating complement, preventing cytokine induced damage, correct coagulation and immunological disturbance

Neonatal Sepsis: Management Principals.

Treatment of Infection	Correction of the consequence of the sepsis process
1. Kill the pathogen (antibiotic)	**C** = Fluids: Colloid/Crystalloid
2. Inhibit/Reduce toxin levels	Maintain perfusion/volume
3. Regain immunological balance	Early inotropic support
	H = Transfusion, Vitamin K
	A = Maintain pH, glucose
	Maintain tissue oxygenation by
	Early ventilation if required
	Nutrition
	O = Symptomatic organ support
	Nutrition
	S = Correct early immune paralysis

Fig. (2). Management principals of neonatal sepsis.

all at the same time! It is clear therefore, that there is unlikely to be a single 'magic bullet' that could cover all these requirements. Hence a composite generic 'package' or 'bundle' of care needs to be developed that could be adapted to the particular and unique needs of an individual baby. Recommendations offered below are based on evidence where available, personal practice and pragmatism; they cannot replace the wisdom of an experienced clinician who has to make clinical decisions 24/7 based on the available unique set of variables for any given infant.

Initial Resuscitation

Initial standard resuscitation should be initiated as soon as it is recognised that the infant has severe sepsis or impending septic shock (*Fig. 1*) which is often difficult to recognise early.

Killing the Pathogen (Antibiotic Therapy)

There is strong evidence that intra-partum prophylaxis for GBS or preterm prolonged rupture of membrane reduces the risk of neonatal infection.[47,48] Use of Ampicillin instead of penicillin for GBS prophylaxis has raised concerns about the rise of Gram-negative and Ampicillin resistant *E.Coli* infections.[49,50]

Knowledge of local flora and different characteristics of antibiotics are key to effective and safety of antibiotic therapy. In clinical practice, threshold for starting antibiotics on suspicion of infection is justifiably diffuse and low. There is almost universal agreement that initiating early empiric antibiotic treatment on suspicion of EOS with penicillin (or a penicillin derivative like Ampicillin) plus an aminoglycoside (frequently Gentamicin) after obtaining adequate cultures and other samples is important because delay in initiating antimicrobial therapy is known to worsen the outcome.[51] Choice of antibiotic however depends on the known susceptibility pattern, but should have a wide spectrum and be bactericidal in nature. Some initiate mono-therapy with a second or third generation cephalosporin in extremely low birth weight infants due to their relative lack of toxicity and better concentrations in the CSF. It must be emphasised

that use of wide spectrum mono-therapy with third generation cephalosporins has been associated with rapid development of glyco-peptide resistant enterococci and selection of beta-lactamase producing Gram-negative organisms.[52] Evidence from randomised clinical trials however does not suggest that any one regimen of antibiotic/s is superior to another in either EOS or LOS.[52,53] Initial antibiotic therapy should be altered on the basis of microbiological and clinical data once a pathogen/s have been identified.

As stated above no ideal regimen for treating suspected LOS can be recommended either; many clinicians use a combination of glycopeptides (Vancomycin or Teicoplanin) and Ceftazidime (or an aminoglycoside) initially. For suspected Gram-positive Coagulase Negative (CONS) organisms like or *S. epidermidis* antibiotic regimens consisting of either Vancomycin alone or Vancomycin and an aminoglycoside or Tichoplanin is suggested as most strains of *S.aureus* produce beta lactamse making them resistant to penicillin G, Ampicillin, Carbenicillin and Ticarcillin.

Mono therapy with Vancomycin should be avoided due the potential of developing Vancomycin insensitive (VISA) or resistant (VRSA) *S. aureus,* and Vancomycin resistant *enterococci* (VRE). Cephalosporins are an attractive alternative due their lack of toxicity and good CSF concentration but their use has been associated with increase in resistance by Gram-negative organisms e.g. *Klebsiella pneumoniae*.[54] Nafcillin or oxacillins are other useful substitutes to Vancomycin.

For Gram-negative organisms either third generation cephalosporin or an aminoglycoside (Gentamicin or Amikacin) are the usual antibiotics of choice. Emergence of resistance in Gram-negative organisms to these antibiotics is a cause for concern.[54,55] Aminoglycosides and Vancomycin are both potentially nephrotoxic and ototoxic, therefore should be used with caution and their serum levels monitored.

Extended spectrum beta lactamase (ESBL) producers like *Klebsiella, Serratia and E.Coli* are resistant to ß-lactam agents. These organisms are best treated with carbepenems (Meropenem, Imipenem) with or without fourth generation Cephalosporins e.g. Cefepime.[56] Meropenem is preferred over other carbapenems in neonates because of its better safety profile.[57] Aztreonam a mono-lactum which is tolerated well by neonates is effective against antibiotic resistant Gram-negative bacilli and aerobic Gram-negative bacilli.[58,59]

Whilst the choice of antibiotics used is determined by susceptibility of the pathogen to the antibiotic and its pharmacokinetics, there is no consensus as to the duration of antibiotic therapy. Most clinicians stop antimicrobial therapy if the blood culture is negative and the infant is well. In culture proven sepsis clinician often give a 'course' which varies from 5 to seven to 21 days (mean 9 days),[60,61] longer for meningitis (14-21 days)[62] and osteomyelitis (4-6 weeks). These 'courses' are not based on any evidence but dogma and personal practice. Except for meningitis and osteomyelitis there is ample evidence that shorter duration of antibiotic therapy (5 days or less) in culture proven sepsis is either as good or better than giving antibiotics for longer periods.[60-65] Recently Engle and colleagues have shown that cure and recurrence rates in term infants with pneumonia were the same between those infants who received antibiotics for four days or seven days.[66] This is supported by Marc Labenne and colleagues[67] who found that reducing duration of antibiotic therapy does not increase the risk of infection relapse in neonates with early onset sepsis, in fact it decreased the incidence of late onset sepsis in these infants. For the last fifteen years it has been our practice not to give antibiotics for more than four days in culture proven sepsis (except in meningitis or osteomyelitis) and have had no cause to regret this practice. Ideally duration of antibiotic therapy should be guided by infants clinical condition

and biomarkers like IL-8, MIP-1ß, or PCR. Where these are not available then a combination of C-reactive protein and immature to total neutrophils ratio provide a good monitoring tool with a NPV of 94% (*Table 2*).

There are no studies comparing different durations of antibiotic treatment in neonatal meningitis thus it is difficult to give evidence based guidance. Our practice is to give antibiotics for two weeks for Gram-positive and three weeks for Gram-negative meningial infection. Textbook recommendation for duration of antibiotic therapy for osteomyelitis is six weeks but recently shorter periods of antibiotics (2-3 weeks) have been advocated with good results.[68,69]

Intravascular access devices are a major source of sepsis; they should be promptly removed if thought to be infected. Prophylactic antibiotic (low dose Vancomycin) have been shown to have some benefit[70] but the potential of developing either Vancomycin resistant (VRE) or Vancomycin insensitive *S. aureus* (VISA) heavily out weighs the benefit. We do not endorse the use of prophylactic Vancomycin.

Adjunctive Therapy

Neonates more than any group of patients are likely to be exposed to prolonged use of broad-spectrum antibiotics thus are vulnerable to multi-resistant pathogens. Moreover, despite the dramatic increase in both the number of novel antimicrobial agents, antibiotics have proven to be alarmingly insufficient on their own to combat infections in these vulnerable infants with the added problem of increasing drug resistance. This has generated considerable interest in the development of adjunctive immune-modulatory therapies. It is recognized that some antimicrobial agents also have immune-modulatory effect either by directly effecting the immune response or as an indirect consequence of the release of immune modulatory molecules from the bacteria or host cells e.g. depression of phagocytosis (*aminoglycosides*), anti-inflammatory (*macrolides*) while some cephalosporin's enhance immune function but β lactams have no known immune-modulatory effect.

Methods to physically remove toxins by *exchange transfusion* or replace the depleted storage pool of neutrophils by *granulocyte transfusion* have not been successful (RR 0.89, 95% CI 0.43, 1.86) in reducing mortality from sepsis and significant pulmonary complications have been reported following granulocyte transfusion.[71]

Haemopoetic growth factors (GM-CSF, G-CSF) have also not been shown to reduce mortality from sepsis (RR 0.71, 95% CI 0.38, 1.33) except in infants who along with sepsis have severe neutropenia and are growth restricted (RR 0.34, 95% CI 0.12, 0.92).[72] Use of TNFα antibodies, soluble TNF receptor, IL-1ra bacterial permeability increasing proteins or nitric oxide inhibitors have failed to reduce mortality from sepsis.[73]

Activated protein C which reduces neutrophils adhesion to vascular endothelium and restricts TNF α, IL-1 and IL-6 secretions from monocytes has been found useful in adults but in neonates significant bleeding has been reported with its use. Thus, its use is not recommended until further studies in the newborn are available. Low dose steroid therapy has not been investigated in the newborns nor has therapy with pro-inflammatory cytokine INFγ.[74,75]

Pentoxifylline: A xanthine derivative, carbonic anhydrase inhibitor that inhibits release of TNFα and improves white cell function has been shown to significantly reduce mortality in infants with sepsis in small studies from Poland (RR 0.14, 95%CI 0.03, 0.76).[76] Larger trials of this exciting drug are underway in neonates.

Polyclonal Intravenous Immunoglobulin (IVIG): By virtue of their diverse repertoire immunoglobulin's posses a wide spectrum of antibacterial and antiviral specificities.[77] IVIG provide antimicrobial efficacy independently of pathogen resistance. While individual

clinical trials of IVIG in neonatal sepsis have demonstrated dramatic reduction in mortality, number of systematic reviews have yielded contradicting results, in part due to 1) varying study design, 2) failure to include important studies in analyses, 3) inclusion of neonatal, paediatric and adult studies and 4) combining prophylactic and treatment studies together. In our view IVIG continues to represent one of the most promising adjuvant strategies for the treatment of infection in both adults and neonates[78] for the following reasons; Whilst IVIG are polyclonal and heterogeneous serum/plasma derived agents making each preparation distinct and unique. They on the whole have been shown to; increase the number of circulating neutrophils, improve neutrophil migration to the site of infection, prevent depletion of neutrophil storage pool in neonates, increase neutrophil chemo luminescence, opsonic activity, and chemotaxis while also activating complement and inactivating C3b containing complexes thereby reducing C3 activation and complement mediated inflammation.[5] Kazatchkine[79] has shown that IVIG modulate antibody and cytokine production and activation, interfere with selection of B cell repertoire, control B cell proliferation, neutralize pathogenic auto antibodies, regulate CD8 mediator suppressor or cytotoxic T cell function and super antigens. IVIG down regulate the IL-1 system; contain antibodies directed against IL-1, IL-6 and IFN α, β, and γ that modulate the cytokine cascade. IVIG's have also been shown to have cytoprotective effect on TNFα induced cell death in fibroblasts.[80-87] Thus there are many good reasons to consider the use of IVIG in the treatment of sepsis particularly in critically ill and or immune-compromised patients like the VLBW infant.

In a Cochrane systematic review significant reduction in mortality was noted in infants with proven sepsis (RR 0.55, 95% CI 0.31, 0.98).[88] In two recent meta-analyses addition of IgM-enriched IVIG to standard treatment has also shown highly significantly reduction in mortality from sepsis (RR 0.35, 95% CI 0.23, 0.54)[89] and (RR 0.50, 95% CI 0.34, 0.73)[90] thus, it would seem adding IVIG as adjunct to standard therapy is advantageous. Complication rates reported are extremely low (<0.5%) and mandatory regulations have made IVIG one of the safest blood products available but the extremely low risk of viral or prion transmission of 1/4800, 000, 000 cannot be totally excluded. Recruitment to the International Neonatal Immunotherapy Study (INIS) that has been looking whether addition of polyclonal IVIG to standard treatment reduces mortality in neonatal sepsis has just finished and results of this study are awaited. (http://www.npeu.ox.ac.uk/inis/index.php).

Bovine Lactoferrin: In a recent randomized trial bovine lactoferrin supplementation has been shown to reduce the incidence of late onset sepsis in VLBW infants (risk ratio, 0.34; 95% CI, 0.17-0.70; p = .002)[91] but further studies are required to confirm these results.

Supportive Therapy

Fluid Therapy: Fluid resuscitation is the hallmark of treating hypovolemic and septic shock. It does not matter whether colloid or a crystalloid solution is used (though possibly smaller volume of colloid is required for the same effect).[92] There is no evidence from randomized clinical trials to support routine use of early volume expansion in very preterm infants without cardiovascular compromise and insufficient evidence to determine whether infants with cardiovascular compromise benefit from volume expansion. However, in sepsis there is often 'third spacing' or pooling of fluid in the vaso-dilated compartment, for which isolated slow bolus of 10-20ml/kg of fluid given over 20-30 minutes may be helpful. To prevent reperfusion injury it may be preferable to increase the total volume of fluid rather than give boluses. End points of adequate fluid resuscitation in sepsis should be normalization of heart rate, oxygen saturation, serum lactate and pH. It is important to remember that those infants who after adequate fluid resuscitation

do not self-diurese may need diuretics to prevent fluid overload.

Inotropes: Fluid resuscitation is key and must be achieved prior to instituting vasopressor or inotropic agents. Dopamine acts by its vasoconstrictive action and dobutamine by increasing cardiac contractibility and output. In neonatal sepsis there is initially fall in vascular resistance due to vasodilatation that is followed by decrease in cardiac output. Hence, dopamine is often the first inotrope chosen. In a systematic review[93] dopamine was found to be marginally more effective in the short term. Clinically however, it does not significantly alter the outcome which inotrope is used first. There is little experience using other vasopressors in neonates with sepsis.

Coagulation: Sepsis causes the vascular endothelium to become prothrombotic and anti-fibrinolytic. In sepsis anti-thrombotic factors are consumed leading to micro-thrombi formation and DIC, followed by consumption of prothrombic factors leading to spontaneous bleeding. It is important therefore to constantly evaluate coagulation profile of the preterm infant with sepsis. Prolonged prothrombin time/ partial thromboplastin time and low fibrinogen levels suggest DIC there is neither consensus nor evidence as to the best method to treat DIC.

Thrombocytopenia is also a feature of severe sepsis and once again there is no consensus when platelet transfusion should be given though most would transfuse platelets if they were less than 50,000/cu mm.[5]

Anaemia: Anaemia is not an uncommon feature in sepsis due to bleeding, haemolysis and blood loss from multiple sampling. There are no studies guiding transfusion policies in septic newborns. As alluded to above, tissue perfusion and oxygenation are often compromised in sepsis, these must be rectified. In our practice we accept a lower limit of haemoglobin of 10 grams/dl (Hct 33) in a septic preterm neonate below which we would transfuse red cells but have no evidence to support this practice.

Metabolic Control: There is insufficient evidence from randomised controlled trials to determine whether infusion of base or fluid bolus reduces morbidity and mortality in preterm infants with metabolic acidosis secondary to sepsis.[94] Acidosis is usually secondary to hypoperfusion or hypoxia that require correction in their own right. Bicarbonate solutions are very hyperosmolar and are associated with intra-ventricular bleeding, thus they should be used sparingly and with caution.

Of greater importance is to maintain a tight glycemic control during sepsis. Hyperglycemia in sepsis by itself is immuno-suppressive and prothrombotic in nature, thus has the potential to make the clinical condition and outcome worse. Hyperglycaemia in sepsis is mainly due to insulin resistance preventing glucose from entering the Kerb cycle. Whilst there is agreement not to allow glucose level to fall below 30 mg/dl, there is no consensus as when to institute insulin therapy. Hyperglycemia is best treated by early initiation of insulin therapy rather than reduction in glucose concentration of infusion. As a matter of good practice rapid fluctuation in blood glucose levels should be avoided.

Nutrition

During sepsis the infant is catabolic (using its own tissue as metabolic fuel) thus its metabolic and caloric needs are increased, this is worse in preterm VLBW infants who has poor muscle mass, body fat and energy reserves. It is essential that catabolic state secondary to sepsis is corrected rapidly by providing the infant with adequate quantities of energy (10% dextrose infusion is adequate to provide 4-8 mg/kg/minute of glucose) minerals, and vitamins.[42] Enteral feeding is preferable as it reduces bacterial translocation from the gut into systemic circulation. If enteral feeding is not possible or additional energy source is required then parenteral nutrition (TPN) should

be provided. It should be remembered that the major factor responsible for TPN-induced bacterial translocation and intraepithelial lymphocytes changes is the lack of enteral feeding and not the administration of the TPN *per se* [95] therefore where ever possible some enteral feed (non-nutritional/trophic) should be provided.

Strategies to prevent Organ function

Lungs: Respiratory failure in severe sepsis or septic shock is common due to acute lung injury caused by infiltration by activated neutrophils, and surfactant consumption leading to rapid fall in functional residual capacity that may require early ventilatory support and surfactant therapy. Care should taken to avoid hyperoxia for fear of retinopathy of prematurity (ROP) and over distention of alveoli which is a potent inducer of IL-6 release predisposing the infant to secondary lung infection i.e. ventilator associated pneumonia.

Kidneys: Ion channels in tubular epithelium are energy/oxygen dependent thus particularly sensitive to hypotension and hypoxia. Two thirds of preterm VLBW infants will develop renal function abnormalities with sepsis these should be looked for and treated conventionally. There is no evidence that renal replacement therapy (haemofiltration or haemodialysis) is of any benefit.

Liver: Liver insult during sepsis is reflected by sharp rise in liver enzymes and worsening coagulation profile. This damage is often self-limiting but should be treated by standard conventional methods.

Gastrointestinal Tract: As alluded to earlier an empty gut may worsen or initiate sepsis due bacterial translocation across inflamed or damaged intestinal mucosa. This is worse in preterm VLBW infants who lack of immunological protection by sIgA. Use of H_2 antagonists and continuous feeding should be avoided during this period as they increase the gastric pH allowing bacteria to pass through this barrier.

Prevention of Infection

Time immemorial adage of *'prevention is better than cure'* is most apt with regard to neonatal sepsis. Principles of preventing infection are universally known and well documented. (http:/www.cdc.gov.htm, http:/www.cdc.gov.mmwr.htm)[96] they include;

a) Obsession regarding hand hygiene.
b) Education and constant re-enforcement of all staff.
c) Avoiding overcrowding.
d) Maintaining adequate nurse/patient ratio.
e) Applying universal precautions on patient contact.
f) Continuous monitoring and surveillance of infection.
g) Closed system of drug delivery.
h) Applying correct disinfectant to clean equipment.
i) Restricting the use of third generation cephalosporins.

Suggested Management Package (Care Bundle)

From the description and evidence provided above it is clear that management of neo-natal sepsis requires a thorough understanding of host defences systems of the preterm VLBW infant and of the sepsis process itself to be able to develop a comprehensive package of care.

Such a care package is presented below;

1) Early recognition of sepsis (Risk factors $\pm$ signs and symptoms).
   Evidence Class A
2) Early institution of appropriate sepsis screen (inclusion of PCR and cytokine measurements if available).
   Evidence Class A
3) Early initiation of appropriate antibiotics (consider shorter duration therapy).
   Evidence Class A

4) If perfusion is poor AND serum lactate > 5 mmol/l give 10-20 ml of colloid, if still poorly perfused or hypotensive start inotropes. Evidence Class B

5) Maintain Haemoglobin > 10G/dl (Hct ≤ 33). No Evidence

6) Maintain caloric intake > 100Kcal/day entrally or > 80 Kcal/day if on TPN add some trophic feeding if possible. Evidence Class B/C

7) Maintain oxygen saturation between 90 and 92%. Evidence Class A

8) Consider adjuvant IVIG (IgM-enriched) therapy. Evidence Class B

Conclusion

It is recognised that while this review is long and static i.e. it presents evidence as we understand it today and sepsis is a dynamic process. Our understanding, ability to diagnose and manage neonatal sepsis is constantly changing and will continue to change and evolve. By presenting this review it is hoped that practices would become rationale, evidence based and dogma abandoned.

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