PREDICTIVE MODEL FOR DIAGNOSIS OF NEONATAL SEPSIS

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PREDICTIVE MODEL FOR DIAGNOSIS OF NEONATAL SEPSIS

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PREDICTIVE MODEL FOR DIAGNOSIS OF NEONATAL SEPSIS

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ABSTRACT

Early diagnosis of neonatal sepsis is essential to prevent severe complications and avoid unnecessary use of antibiotics. The objective of the study was to develop a predictive model for the diagnosis of neonatal sepsis.

This case-control study was conducted in Queen Sirikit National Institute of Child Health, Bangkok, Thailand. Data were derived from the medical records of 45 sepsis and 135 non-sepsis neonates for early sepsis, and 52 sepsis and 156 non-sepsis cases for late sepsis, during the period 1 October 2004-30 September 2007. Only proven neonatal sepsis cases were included in the sepsis group. Potential predictors consisted of risk factors, clinical conditions, laboratory data, and treatment modalities. The models were developed based on multiple logistic regression analysis.

The incidence of early and late proven neonatal sepsis was 1.27 and 1.46%, respectively. For early neonatal sepsis, the equation and score consisted of 5 variables: length of stay pre-sepsis, poor feeding, abnormal oxygen saturation (<92%), thrombocytopenia (<150 000/mm^3), and leucopenia (<5000/mm^3). For late neonatal sepsis, the model had 6 variables: poor feeding, abnormal heart rate (outside the range 100-180 x/min), abnormal temperature (outside the range 36^o-37.9^oC), abnormal oxygen saturation, abnormal leucocytes(according to Manroe's criteria by age), and abnormal pH (outside the range 7.27-7.45). The area below the Receiver Operating Characteristics (ROC) curve were 87.8 and 95.5% for early and late neonatal sepsis, respectively. Validation used subsets of the original data-set, twice for each model, and produced areas below the curve of 82.2 and 86.2% (for the early group) and 96.3 and 93.6% (for the late group). For early sepsis, score 1 had a sensitivity of 73.3% and specificity of 84.4%. For late sepsis, score 2 had a sensitivity of 88.5% and specificity of 90.4%.

In conclusion, 2 predictive models were developed, one for proven early-onset and another for proven late-onset neonatal sepsis. Derivation and preliminary validation produced good results.

KEY WORDS : PREDICTIVE MODEL/EARLY ONSET NEONATAL SEPSIS/ LATE ONSET NEONATAL SEPSIS/ DIAGNOSTIC TOOL/ SCORING SYSTEM/THAILAND

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LIST OF ABBREVIATIONS

ABBREVIATION OR SYMBOL	TERM
AGA	Appropriate for Gestational Age
ALT	Alanine aminotransferase
ANC	Ante Natal Care
AST	Aspartate aminotransferase
AUC	Area Under The Curve
BE	Base Excess
BGA	Blood Gas Analysis
BP	Blood Pressure
BPD	Bronchopulmonary Dysplasia
BSI	Blood Stream Infection
BUN	Blood Urea Nitrogen
BW	Body Weight
^{o}C	Degree Centigrade (Celcius)
C	Complement
CBC	Complete Blood Count
CD	Cluster Differentiation
CFU	Colony Forming Unit
CONS	Coagulase Negative *Staphylococcus*
CRP	C Reactive Protein
CSF	Cerebrospinal Fluid
DD/MM/YY	Date/Month/Year
dL	Deciliter
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
Eg	For example

LIST OF ABBREVIATIONS (Continued)

ABBREVIATION OR SYMBOL	TERM
ELBW	Extreme Low Birth Weight
EOS	Early Onset Sepsis
ESR	Erythrocyte Sedimentation Rate
et al	And others
FBE	Full Blood Examination
FIRS	Fetal Inflammatory Response Syndrome
g	Gram
GBS	Group B *Streptococcus*
H_2O	Water
HAI	Healthcare Associated Infection
H. influenzae	*Haemophilus influenzae*
hpf	High Power Field
hr	Hour
IAP	Intrapartum Antibiotic Prevention
ie	That is
IL	Interleukin
IM	Intramuscular
IMCI	Integrated Management of Childhood Illness
I/T ratio	Immature / Total ratio
IV	Intravenous
IVH	Intraventricular Hemorrhage
kg	Kilogram
LBW	Low Birth Weight
LFT	Liver Function Tests
LGA	Low for Gestational Age
L. monocytogenes	*Listeria monocytogenes*
LOS	Late Onset Sepsis

LIST OF ABBREVIATIONS (Continued)

ABBREVIATION OR SYMBOL	TERM
LR	Likelihood Ratio
mg	Miligram
min	Minute
MKD	Miligram/Kilogram body weight/Day
ml	Mililiter
mm	Milimeter
mm^3	Cubic Milimeter
MRSA	Methicillin Resistant *Staphylococcus aureus*
mo	Month
NEC	Necrotizing Enterocolitis
NICU	Neonatal Intensive Care Unit
N. meningitidis	*Neisseria meningitidis*
NPV	Negative Predictive Value
O_2	Oxygen
OD	Once Daily
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PDA	Patent Ductus Arteriosus
pg	Picogram
PIRO	Predisposition, Insult Infection, Response, Organ Dysfunction
PPV	Positive Predictive Value
PRM	Premature Rupture of the Membrane
q	Every
QSNICH	Queen Sirikit National Institute of Child Health
RDS	Respiratory Distress Syndrome

LIST OF ABBREVIATIONS (Continued)

ABBREVIATION OR SYMBOL	TERM
RFT	Renal Function Tests
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristic
ROM	Rupture of the Membrane
s	Second
S. aureus	*Staphylococcus aureus*
SD	Standard Deviation
SE	Serum Electrolyte
SGA	Small for Gestational Age
SIRS	Systemic Inflammatory Response Syndrome
S. pneumoniae	*Streptococcus pneumoniae*
STI	Sexually Transmitted Infection
TNF	Tumor Necrosis factor
TPN	Total Parenteral Nutrition
uL	Microliter
U	Unit
USA	United States of America
UTI	Urinary Tract Infection
VEOS	Very Early Onset Sepsis
VLBW	Very Low Birth Weight
WBC	White Blood Cell
WHO	World Health Organization
wk	Week
yr	Year
%	Percent
>	More than

LIST OF ABBREVIATIONS (Continued)

ABBREVIATION OR SYMBOL	TERM
≥	More than or equal
<	Less than
≤	Less than or equal

CHAPTER I
INTRODUCTION

1.1. BACKGROUND AND SIGNIFICANCE OF THE PROBLEM

Over 130 millions babies are born every year and around 4 millions infants die in their neonatal period. Among them, around 1.6 millions die because of infection, in developing countries only. Most deaths in the neonatal period all over the world occur in the first few days after birth. Ninety eight percent of neonatal deaths occur in developing region (33 per 1000 live births), 28% in the least developed countries (42 per 1000 live births).(WHO 2006) Sepsis and meningitis are responsible for most of these infection-related deaths.(Vergnano et al., 2005) In hospital, rule out sepsis is one of the most common discharge diagnosis in neonatology.(Escobar et al., 2000; Gerdes 2004)

Among all newborns, sepsis occur particularly in preterm, low birth weight infants.(Bizzaro et al., 2005) Despite advances in neonatal care overall case fatality rates from sepsis range from 2% to as high as 50%.(Klein 2001) It is obvious that sepsis is a major health problem among children in both developed and developing countries.(Gerdes 2004; Remick 2007)

Early diagnosis (and treatment) of the newborn infants with suspected sepsis are essential to prevent severe and life threatening complications. In the era of multi drug resistance, it is also mandatory to avoid unnecessary use of antibiotics to treat non-infected infants. Thus, rapid diagnostic tests that differentiate infected from non-infected infants, particularly in the era of newborn period, have the potential to make a significant impact on neonatal care.(Mishra et al., 2006)

In the United States alone each year some 600 000 infants will be evaluated for bacterial infection and as many as 400 000 infants will be treated with antibiotics during the birth hospitalization. This can cause alteration in the composition of endogenous flora, infection with multiple drug resistant bacteria, longer hospital stays, and higher costs. In infants and young children, alterations in the microflora

may lead to opportunistic infections and importantly impact subsequent immune development.(Schelonka, 2007)

The unnecessary treatment of babies with suspected sepsis also presents a problem in developing countries with limited hospital facilities and budgets. Any measure to reduce the duration of antibiotic therapy and hospitalization in these infants would therefore be of merit.(Hlwelekazi et al., 2000)

To date, however, no single biological infection marker has gained unanimous acceptance.(Gerdes 2004; Fischer 2005) Identification of the proper information from the history, and the clinical signs and symptoms that are most predictive of the presence of severe acute illness in neonates is needed to devise the accurate case definition and effective case management protocols.(Darmstadt et al., 2000) So far, various strategies to reduce mortality and morbidity in newborns with sepsis involve the use of combinations of clinical signs with hematological and serological markers for identification and intervention in babies at risk. Unfortunately, clinical signs are non specific and often manifest themselves in the absence of a positive culture.(Mishra et al., 2006)

The choice of which variables are to be included in the score made of combination of demographic, physiological, and clinical data, and their relative weights, is obviously vital. A balance needs to be drawn between a complex score including many variables, and therefore difficult to complete, and a simpler model that may be easier to use but not as accurate. It is also need to be remembered that no score can completely quantify the complex factors that make up an individual infant's morbidity.(Dorling et al., 2005) Most of the studies from western countries have placed greater reliance on the value of laboratory test and less emphasis on the clinical signs. Laboratory facilities to perform such tests are more often unavailable as emergency laboratory, and clinical decisions are more commonly made without them.(Okascharoen et al., 2005)

The task of simultaneously considering a number of variables in deciding on the probability of a diagnosis, or the likelihood of a subsequent adverse event, represents a considerable cognitive challenge. In this case, mathematical models that simultaneously consider all relevant variables will do a better job than clinicians' intuition.(Guyatt 2006)

Clinical predictive model (or prediction rule) is a decision-making tool for clinicians that contained 3 or more variables obtained from the history, physical examination, and simple diagnostic tests. They are derived from data collected directly from the patients.(Laupacis et al., 1997) They provide powerful tools to improve clinical decision making.(Reilly and Evans, 2006)

Predictive models quantify the relative importance of particular findings when evaluating an individual patient.(Reilly et al., 2006) It attempts to formally test, simplify, and increase the accuracy of clinicians' diagnostic and prognostic assessment and are most likely to be useful in situations where decision making is complex, the clinical stakes are high, or there are opportunities to achieve cost savings without compromising patient care.(McGinn et al., 2000) However, prediction rule should be regarded as one piece of diagnostic information among others and should never be substituted entirely for a careful diagnostic evaluation in each individual case.(Shapiro 2005)

The main treatment of neonatal sepsis is antibiotic. As bacterial infection predominate, empiric antibiotic regimens focus on cover both gram positive and negative bacterial infection. These antibiotics can be either narrow or broad spectrum in the range of organisms that they target.(Gordon and Jeffery, 2005)

1.2. OBJECTIVES

The primary objective of this study is to develop the predictive model for diagnosis of neonatal sepsis.

The secondary objectives are :

1. To describe the clinical and laboratory diagnosis of neonatal sepsis
2. To describe seasonal variation of neonatal sepsis
3. To describe risk factors of neonatal sepsis
4. To describe the treatment pattern of neonatal sepsis

1.3. BENEFITS

The benefits of this study will be :

1. Faster, more accurate and more reliable diagnostic method will give a better treatment

2. Developing a specific diagnostic method in local level can provide better and more suitable results
3. Possibility to use the method in other places also.

CHAPTER II
LITERATURE REVIEW

2.1. DEFINITION

Infection is a continuum, while it is far more common that a given clinical presentation is best described as a point of continuum. Definitions must attempt to reflect this if they are to be clinically useful.(Haque 2007) Some definitions of terms based on several literatures are on Appendix B.

2.2. EPIDEMIOLOGY

Every year more than 10 millions infants die before their fifth birthday. Almost 8 millions died before their first, in which 4 millions on the first 4 weeks. All over the world most neonatal deaths occurred in the first week, mainly in the first day. WHO estimates 98% neonatal death occurred in developing countries. Infection, prematurity, and birth asphyxia are the main causes. For the infection cause, there are around 1.6 millions neonatal deaths only in developing countries. In Asia, neonatal mortality for all causes is around 34 per 1000 live births. (Vergnano et al., 2005; WHO 2006)

Neonatal sepsis may be classified according to time of onset of the disease : early onset sepsis (EOS) and late onset sepsis (LOS). The distinction has clinical relevance as EOS disease is mainly due to bacteria acquired before and during delivery and LOS disease bacteria acquired after delivery (nosocomial or community sources). In the literature, however, there is little consensus as to what age limit apply, with EOS ranging from 48 hours to 6 days after delivery. This makes it difficult to compare studies where cases are grouped into EOS and LOS without further details.(Gerdes 2004; Vergnano et al., 2005; Datta et al., 2006)

In Asia and Africa, the reported incidence of neonatal sepsis varies from 7.1 to 38 and 6.5 to 23 per 1000 live births, respectively. In USA and Australia the range for total EOS and LOS is 6-9 per 1000 live births.(Vergnano et al., 2005)

In England, Bell et al. (2004) found that between 1982-2000 the rate of neonatal mortality decline significantly (for singleton, it was declined by 20-25% between 1982-1990 and 1991-2000) but there was no reduction in neonatal mortality attributed to infection.(Bell, et al., 2004)

In Australia and New Zealand the mortality of infants with birth weight <1500 g because of gram-negative bacillary infection was 24.2%.(Gordon and Isaacs, 2006) In United States the mortality for the same category was 36%.(Stoll et al., 2002)

In Norway, the frequency of late onset sepsis among infants with BW < 1000 g and 750 g were 220 per 1000 patients and 311 per 1000 patients, respectively.(Ronnestad et al., 2005b) The frequency of VEOS and EOS in the same population was 32.5 and 35.5 per 1000 per population.(Ronnestad et al., 2005)

The high rate of severe sepsis in a large study in USA in infants was largely due to neonatal severe sepsis (69.7% of infants). Two third (69.3%) of neonates were LBW and half (52.7%) were VLBW. The annual incidence of severe sepsis in newborns (first day of life) was 3 of 1000 live births. Among newborns, LBW babies had higher hospital mortality than babies of normal birth weight and LBW boys had higher mortality than LBW girls.(Watson et al., 2003)

During 1991-2003 in another large study in USA, the overall rates of EOS have remained stable (15-19 per 1000 live births of infants VLBW), despite the increase in maternal antibiotics during labor. More than half of EOS in 2002-2003 were caused by gram negative organisms, with *E. coli* as the most common.(Stoll et al., 2005) Study for EOS in infants $\geq$ 2000 grams in California revealed 2.2% sepsis cases.(Escobar et al., 2000)

In Yale, sepsis related mortality rate steadily decrease from 87% in 1928 to 3% in 2003.(Bizzaro et al., 2005) For newborns with birth weight <1000 g and <500 g, mortality rates as a result of sepsis (1989-2003) were 17% and 22%, respectively.(Bizzaro et al., 2005) In population based study, also in USA, the population rate (per 1000 live births) of culture proven infection, clinically confirmed infections, infection-related death, and other death in neonates were 1.2, 2.2, 0.2, and 0.2, respectively.(Escobar et al., 2000)

In Israel the incidence of LOS in VLBW (in a study of national cohort) was 30%.(Makhloul et al., 2002)

In India, each year 1.2 millions die during neonatal period. Three fourth occurred during the first week. A study revealed neonatal sepsis, including pneumonia, emerged as the most common cause.(Mathur 2005) The incidence of blood culture positive sepsis was 21.9 per 1000 live births and 94.7 per 1000 NICU admissions. The odds of having sepsis was greater for lower birth weight babies. The overall mortality is 34%, and the highest was in the lowest birth weight group. The sepsis related mortality reported from various studies in India was range from 10% to 65.3%. Report from study of Bhutta et al in Pakistan found a mortality of 42%.(Upadhyay et al., 2006)

Data from Ramathibodi Hospital in Bangkok, Thailand, revealed that in 1998-2000 as many as 21 445 babies were born. Among them 9347 were hospitalized >72 hours and around 5% had suspected LNS. Only 17 of 1824 neonates had positive culture results.(Okascharoen et al., 2005) Another report from Siriraj Hospital said that between 1996-2001, only 19 cases of GBS found. The incidence of late onset disease was 0.05 per 1000 live births in 1996.(Yossuck and Preedisripipat, 2002) There are 1200 neonates patient during the year of 2006 in Queen Sirikit National Institute of Child Health, 298 among them were diagnosed as sepsis patient. Only in 65 cases the culture results were positive. Twenty five neonatal patients died (data from Medical Record Unit QSNICH Bangkok).

In developed countries, EOS is often more severe and fatal than it is for LOS. As the latter is usually caused by CONS, the associated morbidity and mortality are low. In developing countries LOS has a higher case fatality rate particularly when gram negative bacteria are involved.(Vergnano et al., 2005) Preterm infants (<37 completed gestations) accounted for more than two thirds of all neonatal deaths (early and late) from sepsis during 1985-1998.(Lukacs et al., 2005)

For fungi, study in Israel revealed the incidence of acquired fungal sepsis in neonates was 0.4 to 2 cases per 1000 live births and 3.8% to 12.9% of VLBW infants.(Makhoul et al., 2001) Fungal organisms were responsible for 12% of first episode LOS, with *Candida albicans* became the most frequent organism isolated.(Stoll et al., 2002)

2.3. ETIOLOGY

The pathogens most often implicated in developing countries differ from those seen in developed countries. Overall, gram-negative organisms are more common and are mainly represented by *Klebsiella, Escherichia coli, Pseudomonas,* and *Salmonella*. Of the gram-positive organisms, *Staphylococcus aureus*, Coagulase negative *staphylococci* (CONS), *Streptococcus pneumoniae*, and *Streptococcus pyogenes* are most commonly isolated. GBS is generally rare.(Vergnano et al., 2005)

Study by WHO demonstrates that *Streptococcus pneumoniae, Staphylococcus aureus*, and other *Streptococcus* species were might be the most important pathogens in developing countries. In The Philippines, gram negative bacteria were the most common organism isolated.(Quiambao et al., 2007)

In developed countries, neonatal surveillance generally identifies GBS and *E. coli* as the dominant EOS pathogens and CONS the dominant LOS pathogen followed by GBS and *S. aureus*.(Vergnano et al., 2005)

Sepsis after 3 days of age occurred in 21% of VLBW in United States. The vast majority of infections (70%) were caused by gram-positive organism with coagulase negative *staphylococci* accounting for 48% of infections. Rate of infections was inversely related to birth weight and gestational age. The others gram positive are *S. aureus*, *Enterococcus*, and GBS. Gram negative organisms are *E. coli, Klebsiella, Pseudomonas*, *Enterobacter*, and S*erratia*. The mortality rate in gram negative organism was higher, especially the *Pseudomonas*. (Stoll et al., 2002)

In Yale, the relative rise in cases of LOS from commensal species, which have a lower mortality rate than certain species that have dominated neonatal infections in the past, may contribute to decreased sepsis related mortality. In 1979-1988 the overall percentage of infections related to commensal species was 15%, with the majority of infections caused by traditional neonatal pathogens (GBS 37% and *E. coli* 20%). In 1989-2003 commensal species account for 46% of inborn infection whereas the percentage of GBS and *E. coli* has fallen to 12% and 11%, respectively.(Bizzaro et al., 2005) The greater survival of very low birth weight infants has contributed to this shift.(Edwards and Baker, 2003)

The spectrum of organisms responsible for early onset infection (vertically transmitted) differs from those associated with late onset infection (nosocomial).

Most late onset infections in neonatal unit in developed countries are caused by gram positive cocci, with coagulase negative staphylococci the predominant isolates. Although less common, gram negative bacillary infection has a higher mortality.(Stoll, et.al., 2002)

The summary of bacteria as etiologic agents of neonatal sepsis is listed in Table 1. The predominant period of transmission are listed in Table 2.

Table 1. Etiologic Agents of Neonatal Septicemia

BACTERIA	EARLY ONSET	LATE ONSET
Gram positive bacteria		
Group B *Streptococcus (GBS)*	+++	+
Viridans streptococci	+	+
Enterococcus spp.	+	++
Coagulase-negative *staphylococci (CONS)*	-	+++
Staphylococcus aureus	-	+++
Streptococcus pneumoniae	+	+
Listeria monocytogenes	+	+
Gram negative bacteria		
Escherichia coli	+++	++
Klebsiella spp	+	++
Enterobacter spp	+	++
Citrobacter spp	-	+
Serratia marcescens	-	+
Pseudomonas spp	-	+
Salmonella spp	-	+
Haemophilus influenzae	+	-
Neisseria meningitidis	-	+
Other enteric bacilli	+	+
Anaerobic bacteria		
Bacteroides spp	+	+
Clostridia spp	-	+
Others	-	+

Source : Edwards and Baker, 2003

For LOS in VLBW in Israel the gram positive group mainly consisted of CONS, *Staphylococcus aureus*, and *Enterococcus*; meanwhile for the gram negative are *Klebsiella, Pseudomonas, Enterobacter, E. coli*, and *Acinetobacter*.(Makhoul et al., 2002) For VEOS, EOS, LOS in extremely premature infants in Norway the most common causes are CONS, *S. aureus,* GBS, *Klebsiella, E coli*.(Ronnestadt et al.,

2005; Ronnestad et al., 2005b) In a study in Haiti the most common bacteria were *Enterobacter aerogenes*, GBS, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Haemophilus influenzae*, and *Staphylococcus aureus*.(Desinor et al., 2004)

Candida is the most common fungi causing neonatal septicemia (Makhoul et al., 2001; Ronnestadt et al., 2005b) *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis*, found as the commonest cause in Israel (Makhoul et al., 2001) and USA (Benjamin et al., 2006) The somewhat similar result (*Candida albicans*, *Candida parapsilosis*, and *Candida glabrata*) was also found in Baltimore (Shetty et al., 2005) and Torino.(Manzoni et al., 2006) Around 21% of the neonates with candidemia had false negative blood culture. Negative blood culture was also seen for almost one half of the neonates with candidemia meningitis.(Benjamin et al., 2006)

Since there is big discrepancy in the age limitation to categorize EOS and LOS, the data concerning microorganisms might not be precisely grouped. Studies using longer definitions will incorporate larger proportion of cases where the organism acquired horizontally, from nosocomial or community sources, rather than as a result of vertical transmission.(Vergnano et al., 2005)

Table 2. Predominant Time Periods of Transmission of Pathogens in Neonates

BACTERIA	PRENATAL	PERINATAL	POSTNATAL
Group B *Streptococcus*	-	+	+
Enterococcus species	-	+	+
Escherichia coli and other enteric bacilli	-	+	+
Resistant enteric and environmental bacilli	-	-	+
Listeria monocytogenes	-	+	+
Neisseria gonorrhoeae	-	+	-
Treponema pallidum	+	-	-
Coagulase-negative *staphylococci*	-	-	+
Staphylococcus aureus	-	-	+

Source : Modified from Prober 2003

2.4. RISK FACTOR

The risk of sepsis among asymptomatic infants is very low. So, screening, treatment, and observations protocol cannot be based on asymptomatic status

alone.(Escobar et al., 2000) Risk factors can be divided into three groups : maternal, neonatal, and environment (hospital and community). The maternal peripartum risk factors for early onset bacterial infection are listed in Table 3. The lists of risk factors based on previous publications are written on the Appendix C.

Tabel 3. Maternal Peripartum Risk Factors for Early Onset Bacterial Infection

RISK FACTORS	COMMENTS
• Preterm delivery	• Attack rate inversely related to gestational age <37 weeks
• Premature rupture of the membranes	• Rupture of membranes before onset of labor at any gestation
• Chorioamnionitis	• Risk of neonatal septicemia 5-15%
• UTI	• Higher neonatal risk even in asymptomatic mother
• Multiple pregnancy	• Only noted for GBS septicemia
• Prolonged rupture of the membrane	• Attack rate directly proportional of duration of rupture of the membranes >12 hours
• Early postpartum febrile morbidity	• Maternal fever during the first 24 hours postpartum
	• Higher neonatal risk if no prenatal care
• Prenatal care	• APGAR <6 associated with greater risk
• Fetal hypoxia	
• Meconium-stained amniotic fluid	
• GBS bacteriuria during pregnancy	

Source : Edwards and Baker, 2003

Histologic chorioamnionitis is a major predictor of morbidity and mortality in VLBW newborns.(De Felice et al., 2005) Clinical chorioamnionitis includes a temperature greater than 38^{o}C plus 2 of the 5 following signs : WBC > 15 000 cells/mm^{3}, maternal tachycardia >100 beats/minute, fetal tachycardia >160 beats/minute, tender uterus, and foul-smelling discharge. Chorioamnionitis significantly increased the risk of maternal and fetal morbidity. The risk of fetal infection associated with maternal chorioamnionitis is 10-20%. Complications of chorioamnionitis are more common in premature and low birth weight infants.(Newton 2005)

Epidural labor analgesia is allegedly associated with maternal fever and increase in the newborn sepsis work-up rate, although not more likely to cause neonatal sepsis.(Kaul et al., 2001; Goetzl et al., 2001)

In Table 4, Newton (2005) described the neonatal sepsis by gestational age.(Newton 2005)

Table 4. Percentage of Neonatal Sepsis Patients on Different Gestational Age

GESTATIONAL AGE (week)	SEPSIS (%)	GESTATIONAL AGE (week)	SEPSIS (%)
24	25	30	11
25	29	31	14
26	30	32	3
27	36	33	5
28	25	34	4
29	25		

Source : Newton 2005

There are ethnic disparities in the risks of neonatal mortality and morbidity in the NICU.(Claydon et al., 2007) In US African American this disparities was a result of elevated rates of preterm birth, fetal growth restriction, and neonatal sepsis.(Fiscela 2004) Balchin et al. (2007) also found a racial variation between gestational age and perinatal mortality. (Balchin et al., 2007)

Contaminated umbilical cord is another risk factor in which infections can become systemic.(Mullany et al., 2006) Other known risk factor, although it is rare is contaminated breast milk from the mother.(Youssef et al., 2002; Godambe et al., 2005)

In the hospital setting, factors related to healthcare personnel, practices, and the environment are often overlooked and yet remain the most obvious and inexpensive area of intervention. Indeed, the most common route of spread of nosocomial pathogens is person to person transmission within the unit and during transfer of patients between units. The most common iatrogenic factor contributing to neonatal health care associated infection (HAI) is hands of healthcare workers.(Srivastava and Shetty, 2007)

Medical devices such as central venous catheters are commonly used. The nosocomial infection rate was higher in neonates subjected to device use. About 10.8% of catheterized patients developed hospital acquired UTI. The duration of ventilation was also related to the acquisition of HAI.(Srivastava and Shetty, 2007) Some hospital related risk factors are also listed on Appendix C.

Seasonal and temporal variations are already proved to affect incidence of morbidities in rural area. Seasonal variation also has relation with birth weight, preterm deliveries, and hypothermia. However, the relation with incidence of other neonatal morbidities such as sepsis has not been reported.(Bang et al., 2005)

2.5. PATHOGENESIS AND PATHOPHYSIOLOGY

Human neonates and young infants are more vulnerable to infectious agents than older children and adults and are especially susceptible to infections with intracellular pathogen. The heightened susceptibility and the severe course of infections in early life can be attributed, at least in part, to the lack of preexisting immunological memory and competent adaptive immunity. Several innate immune mechanisms are impaired in neonates.(Marodi 2006) The immature immunologic capacities also limit their ability to mount inflammatory responses, resulting in limited expression of signs and symptoms of infection.(Prober 2003)

Infants who experience early-onset sepsis, especially preterm infants, usually have history of risk factors associated with their mother labor and delivery. The pathogenesis is multifactorial and encompasses microbial, host, and metabolic components. Those with heavy maternal inoculum are at greater risk. Premature neonates are at increased risk because of (1) acquisition of only low levels of maternally derived total immunoglobulin / IgG and specific antibody to bacterial pathogen species, (2) immature function of neutrophils and decreased neutrophil storage pools, and (3) immature immune responses to pulmonary invasion and bacteremia. Metabolic factors such as hypoxia, acidosis, and hyperbilirubinemia further compromise host response.(Edwards and Baker, 2003)

Until relatively recently the preponderance of pathobiological mechanisms proposed to explain sepsis has focused on the course that the pathogen has taken in a ravaged bystander-host. The debate over beneficial versus harmful effect of the host

response to infection has persisted. The notion of sepsis as an uncontrolled proinflammatory response gained support with the identification of proinflammatory cytokines. But with the failing of numerous clinical trials involving proinflammatory mediators, it has been proposed that there exists a critical balance between pro and anti-inflammatory mediators, and this balance must be maintained.(Baron et al., 2005; Remick 2007) Brief outlined of the recent sepsis pathway is on Figure 1.(Baron et al., 2005) The figure showed the process was started with infection by either bacterial or non-bacterial pathogen. This infection was followed by some microbial associated molecular patterns. Then, the process would involve some receptors. This figure also showed the role of inflammatory cell response and mediators.

Cellular changes continue the theme of heterogeneity. Some cells work too well such as neutrophils that remain activated for an extended time. Other cellular changes become accelerated in a detrimental fashion including lymphocyte apoptosis. Metabolic changes are clearly present, requiring closed and individualized monitoring. At this point in time no single mediator / system / pathway / pathogen drives the pathophysiology of sepsis.(Remick 2007)

Another thing to be noted is that the process in the late stage of sepsis is not exactly the same with the early one. Late deaths were preceded by the over expression of both proinflammatory as well as anti-inflammatory cytokines, albeit it much lower levels and with more heterogeneity in comparison to the response observed in acute sepsis. And the pattern of late prelethal responses suggest that systemic inflammatory response syndrome to compensatory anti-inflammatory response syndrome transition paradigm fails to follow a simple linear pattern.(Osuchowski et al., 2007)

In the last 10 years, remarkable progress has been made in characterizing the critical role of the host and its interaction with the pathogen in the pathobiology of sepsis. There has been major advancement of our understanding in molecular signaling pathways in response to microbial pathogens, including identification of toll-like receptors, Nod-1 and Nod-2 intracellular pattern recognition receptors, and the peptidoglycan recognition proteins. Additional information is emerging regarding the systemic effect of the inflammatory cell response on numerous target genes that influence cellular and subcellular processes including the microcirculation and mitochondrial function.(Baron et al., 2005)

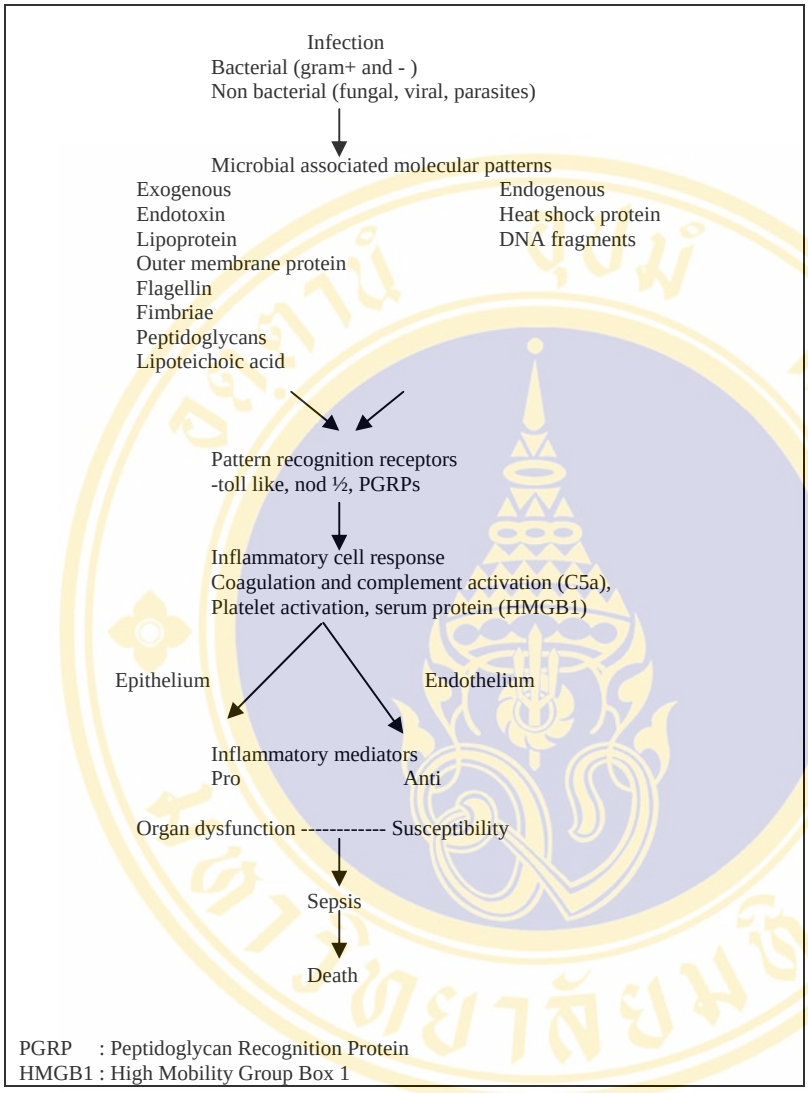

Figure 1. The Recent Sepsis Pathway
Source : Baron et al., 2005

Recently, genetic and immunologic analysis may identify neonates in at high risk of sepsis and could change many epidemiological aspects.(Carrigan et al., 2004; Watson and Carcillo, 2005; Strunk and Burgner, 2006) Several variants in genes involved in the innate immune response have been associated with differential risk for neonatal infection. The most consistent results relate to polymorphisms of tumor necrosis factor-alpha, whereas other gene polymorphisms, such as those of interleukin-6, have yielded conflicting findings. Similar genetic factors may be

involved in other inflammatory neonatal diseases. Recent data suggest that genetic variation may influence the pace of immunologic maturation.(Strunk and Burgner, 2006)

2.6. CLINICAL FEATURES

Clinical signs and symptoms of neonatal sepsis are usually subtle. Even minimal deviations from usual activity should be regarded as possible indications of invasive infection.(Edwards and Baker, 2003; Lott 2006)

There are various guidelines concerning clinical features of neonatal sepsis. Most of them are listed in the definitions of neonatal sepsis. Some others are described below. Presenting signs and symptoms (Bizzaro et al., 2005) : apnea, bradycardia, temperature <36.5^{o}C or >38^{o}C, plus laboratory result : blood glucose <40 or >140 mg/dl.

Clinical symptoms derived from sepsis score : apnea (>20 s), tachypnea (>60/min), bradycardia (five times in 24 h) or persisting tachycardia (>170/min), prolonged capillary refilling (>3s) or poor skin color, hypotension requiring volume or cathecolamine therapy, distended abdomen, neurological symptoms of irritability or lethargy, hepatomegaly, temperature instability (more than 3 different temperatures with changes >0.5^{o}C within 24 h).(Martius et al., 1999)

The clinical features for neonatal sepsis according to Lott (2006), Yossuck and Preedisripipat (2002), Darmstadt et al (2000) and Klein (2001) are listed in Table 5. Clinical criteria according to WHO are listed in Table 6.

A study by Griffin et al (2005) found that heart rate could possibly be used as a novel physiomarker to predict neonatal infection and death. Early in the course of neonatal sepsis there were reduced heart rate variability and transient decelerations similar to fetal distress. Compared with infant with low risk heart rate characteristics measurements, infants with high risk heart rate characteristics measurements had 5-6 fold increased risk for an adverse event in the next day and 3-fold increased risk in the next week. Infants with both high risk heart rate characteristics and abnormal laboratory tests had 6-7 fold increased risk for an adverse event in the next day compared with infants who had neither.(Griffin et al., 2005; Griffin et al., 2005b)

The difference between rectal and sole temperature can also use as a marker of neonatal sepsis. In babies with clinical sepsis, if the rectal sole difference is >2^{o}C, 5.33 times higher probability that the baby has a definite sepsis, and when it is <2^{o}C it is unlikely that the clinical signs are due to sepsis.(Murki et al., 2006) However, in low risk full term infants, fever with no other symptoms during the first days of life (but after the first day) is not primary related with infections.(Maayan-Metzger et al., 2003)

2.7. LABORATORY FINDINGS

Accurate and timely diagnosis of neonatal sepsis remains challenging. The difficulties inherent in determining the diagnosis of bacterial infection in neonates have prompted development of a number of screening tests. For EOS, a test with a rapid turnaround time with 100% sensitivity, rather than high specificity, which allows accurate diagnosis and appropriate antimicrobial treatment or which allows antibiotics to be safely withheld in non infected infants, is desirable.(Edwards and Baker 2003; Mishra et al., 2006)

The ideal early diagnostic test for infection would have 100% sensitivity and 100% specificity. However, such an ideal test is unlikely ever to be discovered, as most tests are measured on a continuous scale where there is overlap between infected and non infected infants.(Mishra et al., 2006) For infection, an infant is likely to suffer if infection is under diagnosed and not treated, than if infection is over diagnosed and the infant is treated unnecessarily. Therefore, the most desirable characteristic for the diagnostic test for neonatal infection is high sensitivity rather than high specificity.(Mehr and Doyle, 2000)

To be able to reduce antibiotic overuse in non infected babies with suspected sepsis, a test with 100% sensitivity will allow safe withholding of antibiotics in babies with suspected sepsis. Even if test is not 100% specific, as long as the specificity is not too low, it will allow some reduction in antibiotic prescribing depending on how low the specificity is and the prevalence of infection in the group of infant at risk.(Mishra et al., 2006)

Currently there is no single laboratory test that has ROC value >0.8. Thus, it remains important for clinician to use a combination of clinical variables and

laboratory test to get the greatest predictive value.(Haque 2005) Some components of basic and non-specific tests are listed in table 7 and the others tests in table 8.

Table 5. Clinical Feature for Neonatal Sepsis

Yossuck and Preedisripipat (2002)-only GBS	**Klein (2001)**
• Fever (t >37.5°C) • Hypotension • Poor perfusion • Cyanosis • Respiratory distress • Lethargy • Apnea • Tachycardia (>160) • Persistent pulmonary hypertension of the newborn • Seizure • Hypoglycemia • Hyperglycemia	• Hyperthermia • Hypothermia • Respiratory distress • Apnea • Jaundice • Lethargy • Anorexia or vomiting • Irritability • Convulsions • Bulging or full fontanelle • Diarrhea or abdominal distention • Hypotension
Darmstadt et al. (2000) • High or low temperature • General: listless, lethargic, difficult to arouse, irritable, excessive or weak cry, inconsolable, ill-appearing • Gastrointestinal findings: poor feeding, vomiting, abdominal distention, diarrhea • Respiratory findings: cough, chest indrawing, rapid breathing, nasal flaring, grunting, noisy or difficult breathing, apnea, ausculatory findings • Neurology findings: decreased/weak suck, full fontanel, hypotonia, decreased spontaneous movements, seizures • Cutaneous findings: altered skin turgor, decreased capillary refill, cyanosis, omphalitis, pustular eruption, jaundice, sclerema neonatorum • Plus risk factors : young age, low weight for age, gender (outcome is poorer for males?)	
Lott (2006) • Skin : Petechiae, sclerema, pustules, jaundice, purpura, cold-clammy to touch • Respiratory : Expiratory grunting, nasal flaring, retractions, tachypnea, apnea • Gastrointestinal : emesis, poor feeding, abdominal distention, residuals, diarrhea, bloody stools, hypoactive bowel sounds • Circulatory/hematopoetic : Decreased perfusion, bradycardia, tachycardia, dysrhythmia, cyanosis, pallor, mottling, temperature instability, edema, hepatomegaly, splenomegaly, thrombocytopenia • CNS : Hypotonia, lethargy, irritability, seizures, full fontanel, abnormal eye movements • Miscellaneous : Hyponatremia, hypocalcemia, metabolic acidosis, hypoglycemia	

Source : Klein 2001, Yossuck and Preedisripipat, 2002; Darmstadt et al., 2003 ; Lott 2006

Table 6. Clinical Criteria for The Diagnosis of Neonatal Sepsis (Severe Bacterial Infection) from WHO

	IMCI CRITERIA FOR SEVERE BACTERIAL INFECTION	WHO YOUNG INFANT STUDY GROUP	WHO MANAGING NEWBORN PROBLEMS
Convulsions	X	X	X
Respiratory rate >60 breaths/min	X	X	X
Severe chest indrawing	X	X	X
Nasal flaring	X	-	X
Grunting	X	-	X
Bulging fontanelle	X	-	-
Pus draining from the ear	X	-	-
Redness around umbilicus extending to the skin	X	-	-
Temperature >37.7^{o}C or <35.5^{o}C	X	X	X
Lethargic or unsconscious	X	X	X
Reduced movements	X	X	X
Not able to feed	X	X	X
Not attaching to the breast	X	-	-
No suckling at all	X	-	-
Crepitations	-	X	-
Cyanosis	-	X	-
Reduced digital capillary refill time	-	X	-
Apnea	-	-	X

Source : WHO Multicenter Study Group 1999; WHO 2000; WHO 2003b

In practical, basic laboratory parameters indicating infections in newborn can include serum CRP >1 mg/dl, neutropenia, a ratio of immature to total neutrophils >0.20 within 72 h after birth. Using CBC results obtained at 12 and 24 hours of age and the use of I:T ratio can classify large number of normal newborns as being at risk for sepsis.(Schelonka et al., 1994; Escobar et al., 2000)

In the neonatal period septicemia can be indistinguishable from meningitis. Lumbal puncture should still be undertaken in babies with severe illness or obviously where meningitis is strongly suspected. There is no need to carry out lumbar puncture in neonates suspected of early sepsis who are being evaluated purely for perinatal risk factors. (Ray et al., 2006)

Table 7. Some Basic Tests for Neonatal Sepsis

TESTS	FINDING SUPPORTING POSSIBLE INFECTION
Total WBC (cells/mm^3)	< 5000 or > 20 000
Total neutrophil (PMN) count (cells/mm^3)	< 4000
Total immature PMN count (cells/mm^3)	> 1100 (cord blood) > 1500 (12 hr) > 600 (>60 hr)
Immature PMNs : total PMNs ratio	> 0.2
Platelet count (cells/mm^3)	< 100 000
C-reactive protein (mg/dL)	> 1.0
ESR (mm/hr)	> 5 (1^{st} 24 hr) Infant's age in days +3 (through age 14 days)
Fibronectin (ug/mL)	20 (>2 wk of age)
Haptoglobin (mg/dL)	< 120-145
Granulocyte colony-stimulating factor (pg/mL)	> 10 (cord blood) > 50 (after delivery) > 200

Source : Edwards and Baker, 2003

Table 8. Some Other Laboratory Tests for Neonatal Sepsis

- WBC count (high or low, immature neutrophil:total neutrophil ratio, neutropenia, toxic granulations in neutrophils)
- Serial CRP
- Chest radiographs
- Oxygen saturation
- Nasopharyngeal aspirate
- Pathogen antimicrobial susceptibility tests
- Serum IL-6, IL-1 alpha
- Plasma IL-1 receptor antagonist
- Soluble IL-2 receptor
- Plasma granulocyte-CSF
- Unique small subunit ribosomal RNA sequences identified by PCR
- Blood culture, CSF culture

Source : Darmstadt et al., 2000

A review by Mishra et al (2006) divided the diagnostic method based on the time into : antenatal diagnosis and post natal diagnosis. Antenatal diagnosis could be done by using (beside the clinical signs and symptoms of the mother) : amniotic fluid TNF alpha, IL-1 beta, IL-6, IL-8, PCR, culture, and proteomics (in the future).

Postnatal diagnosis comprised of several examinations such as : routine investigations, acute phase reactants, cell surface markers, granulocyte colony stimulating factor, cytokines, and molecular genetic.(Mishra et al., 2006).

Routine investigations : based on CBC. Acute phase reactants : CRP and procalcitonin. Alpha 1 antitripsin, fibronectin, haptoglobin, lactoferrin, neopterin, and orosomucoid have also been evaluated. Although most markers show significant increases in infected infants, none have been routinely used clinically, either because of their limited diagnostic accuracy or because they have been superseded by better and more sophisticated tests.(Ng 2004; Ng and Lam, 2006; Mishra et al., 2006)

CRP is synthesized within 6 to 8 hours of exposure to infective process or tissue damage, with a half life of 19 hours and may increase 100-fold during an acute-phase response. The ranges for sensitivity and specificity for diagnosis of early onset sepsis ranges are 43-90% and 7-78% respectively. The specificity and PPV ranges from 93-100% in late onset sepsis. Thus CRP is specific but late marker of neonatal infection.(Mishra et al., 2006)

Serial CRP measurements have an excellent negative predictive value for the presence of infection but the reverse does not hold true. The positive predictive value for CRP is low, especially for culture-proved early onset infection. Moreover, caution should be exercised when applying this approach to infants <34 weeks gestational age.(Hlwelekazi et al., 2000)

Another acute phase protein is serum amyloid A. In comparison with CRP, serum amyloid A level rose earlier and in a sharper manner, had higher levels and returned faster to normal values in infants with early onset sepsis. The sensitivity, specificity, PPV, and NPV were 96, 95, 85, and 99%, respectively.(Arnon et al., 2007)

Lipopolysaccharide binding protein is also a potential marker in the early diagnosis of invasive bacterial infection in children. Follow up serum values of LBP were persistently elevated despite adequate antibiotic treatment, whereas TNF alpha and IL-8 concentrations decreased.(Ubenauf et al., 2006)

Procalcitonin produced by monocytes and hepatocytes which begin to rise 4 hours after exposure to bacterial endotoxin, peaking at 6-8 hours, and remaining raised for at least 24 hours with a half life of 25-30 hours. Several studies have shown

that serum procalcitonin concentrations increase in systemic bacterial infection with sensitivity and specificity ranging from 87-100%. It may be useful in assessing the severity of infection, following the progress of treatment, and predicting outcomes. However, it is not a readily available diagnostic assay in most institution.(Mishra et al., 2006) In a study in children, procalcitonin can differentiate sepsis and SIRS, meanwhile CRP cannot.(Arkader et al., 2006)

Cell surface markers : Neutrophil CD 11b and CD 64 appear to be promising. CD 11b is a subunit of the b2 integrin adhesion molecule, normally expressed at very low concentration on the surface of non-activated neutrophils. The sensitivity and specificity are 86.3-100% and 100% respectively. CD 64 has 97% sensitivity and 90% specificity with 99% NPV. None of these white cell surface markers are readily available diagnostically.(Ng 2004; Mishra et al., 2006; Ng and Lam 2006)

Granulocyte colony stimulating factor is a mediator produce by bone marrow, facilitates proliferation and differentiation of neutrophil.

Cytokines : TNF alpha, IL-1, IL-6, Il-8. In response to antigen such as bacterial endotoxins, activated tissue macrophages produce TNF and IL-1.These proinflammatory cytokines stimulate endothelial cells to express receptors for intercellular adhesions molecule on WBC. This initiates the cytokines cascade towards increase production of IL-6, IL-8, and chemokines. Some bacteria activate epithelial cells directly to produce inflammatory cytokines. Newborn infants display a higher percentage of IL-6 and IL-8 positive cells than adults. On exposure to bacterial products, IL-6 rise sharply precedes the CRP. IL-6 has sensitivity and specificity of 89 and 91%. The sensitivity is reduced at 24 and 48 hours because the concentrations fall rapidly. IL-8 predominantly produced by monocytes, macrophages, and endothelial cells, with similar kinetic to IL-6. The sensitivity range is from 80 to 91% and the specificity from 76 to 100%. TNF alpha is a proinflammatory cytokine that stimulates IL-6 production an has a broad spectrum of biological actions on several types of target cell, both immune and non immune.(Carrigan et al., 2004; Mishra et al., 2006; Ng and Lam, 2006)

Another group of proinflammatory cytokines often linked with sepsis is IL-1 family, including IL-1 alpha, IL-1 beta, and IL-1 receptor antagonist (IL-1 ra). Other markers (adhesion molecules such as : intercellular adhesion molecule 1, vascular

cell adhesion molecule 1, E-selectin, L-selectin ; complement activation products such as : C3a-desarg, C3bBbP, sC5b-9) require further evaluation.(Mishra et al., 2006)

Molecular genetics : broad range PCR analysis use the bacteria specific 16s rRNA gene which is highly conserved in all bacterial genomes. Molecular biology techniques offer the prospect of rapid identification of both pathogens and antimicrobial resistance markers.(Ng 2004; Mishra et al., 2006) With the development of molecular methods over the last twenty years, it has become possible to identify bacteria through analysis of their ribosomal RNA genes (rDNA). This approach yields results, even if species cannot be grown in culture. It should be noted that around 30% of patients with sepsis will have negative cultures. With the use of this method, in fact, it has become increasingly clear that the majority of existing microorganism in the environment cannot be cultured with standard techniques.(Baron et al., 2005) Amplification targeting on this 16s rRNA gene is potentially valuable clinical tool in samples with low copy numbers of bacterial DNA, as this gene is present at 1 to more than 10 copies in all bacterial genomes. The gene also has a number of divergent regions nested within it, so PCR can targeted for species specific detection of bacteria in clinical samples. However the performance is still challenging in the diagnostic laboratory and this detection does not provide antimicrobial sensitivity pattern. Moreover, the success of this approach must be proven on a much larger scale using multiple sites.(Jordan et al., 2006; Mishra et al., 2006)

The greatest predictability usually results from combinations of assays. The list of sensitivity, specificity, NPV, and PPV of some tests used for neonatal sepsis is in Table 9.

Despite favorable claims by many studies, most diagnostic markers fail to meet the stringent demands required for clinical practice. Cost, availability of specimens at the appropriate time, complexity of the assay methods, laboratory turnover time, reliability of the tests, and attitude of attending clinicians are all important factors in determining the suitability of a diagnostic marker for clinical application.(Ng 2004)

Table 9. Sensitivity, Specificity, Positive and Negative Predictive Value of Some Laboratory Tests Used in The Diagnosis of Infection in The Newborn

TEST	SENSITIVITY	SPECIFICITY	PPV	NPV
• Blood culture	11-38	68-100	90-100	72-100
• WBC <5000, >30 000	17-90	31-100	50-86	60-89
• I/T Ratio >0.02	81	45	23	92
• CRP >10 mg/dL	37	95	63	87
• IL-8 >70 pg/mL	77	76	42	94
• I/T ratio >0.02 + CRP >10 mg/dL	89	41	24	94
• IL-8 >70 pg/mL + CRP >10 mg/dL	91	74	43	98
• 16S PCR	96.0	99.4	88.9	99.8
Antenatal				
• Amniotic TNF alpha ≥41 pg/ml	82	79	47	95
• PCR for genomic DNA in amniotic fluid	100	100	100	100
Postnatal				
• CRP	60-82	93-96	95-100	75-87
• CRP, FBE, gastric aspirate	97 82-100	61 87-100	53 86-98	98 93-100
• Procalcitonin	96-100	81-100	22-100	100
• CD 11b	64-97	72-96	64-88	84-98
• CD 64	81-97	71-87	63-74	86-98
• CD 64, IL-6, or CRP	95	73	40	99
• GCSF ≥200 pg/ml	87-90	93	93	93-100
• Umbilical cord IL-6	67-89	89-96	84-95	77-91
• IL-6	93	88-96	86-95	95
• IL-6 and/or CRP	80-91	76-100	70-74	91-95
• IL-8	80	87	68	93
• IL-8 and/or CRP	100	100	100	100
• PCR for genomic DNA in blood culture				

Source : - Textbook of Paediatrics, 6th edition, Mc Intosh N, Stenson B (Eds), Edinburgh, Livingston, 2003. Quoted from (Haque 2005)
- Mishra et al., 2006

Many new chemokines, antimicrobial peptides, acute phase reactants, and cell surface antigens such as epithelial neutrophil activating peptide-8, human beta defensin 1 and 2, calprotectin, and several others are also being investigated.(Ng 2004)

The detection of bacterial antigen in blood, urine, and CSF confirms the presence of systemic bacterial disease. Diagnosis technique includes countercurrent immunoelectrophoresis, latex particle agglutination, and coagglutination procedures. Countercurrent immunoelectrophoresis is specific but has low sensitivity and can be used to detect infections caused by E coli K1 and Group B *Streptococci*. Latex particle agglutination tests are more sensitive than is countercurrent immunoelectrophoresis but they have been associated with a small percentage of false-positive and false-negative reactions. They can be used for the detection of disease caused by Group B *Streptococci*, *N. meningitidis*, *S. pneumoniae,* and *Haemophilus influenzae type b*. The highest yield is achieved by testing concentrated heat-treated urine specimens and CSF. The sensitivity of this test is 90-98%, with an average false positive rate of 2-6%.(Saez-Llorenz and McCracken Jr, 2004)

Direct examination of gram-stained or methylene blue buffy coat smears can help in the early detection of neonatal bacteremia if bacteria engulfed by neutrophils are visualized. Some physicians consider only those smears with intragranulocytic bacteria to be positive. Bacteria are seen more readily when acridine orange stain is used.(Saez-Llorenz and McCracken Jr, 2004)

Endotoxin elaborated from gram-negative bacteria circulates in blood and is present in urine for considerable periods after these fluids have been sterilized. Detection of endotoxin by the limulus amebocyte lysate assay may be helpful in the early identification of infected infants. Endotoxin may be present in blood of septic-appearing infants who have sterile blood cultures. Transient endotoxemia possibly is responsible for “clinical sepsis” in these infants. The source of endotoxin is may be the gram-negative bacterial flora of the bowel. Endotoxin possibly enters the circulation through an injured and permeable gastrointestinal mucosa. (Saez-Llorenz and McCracken Jr, 2004)

2.8. CULTURE

For some clinicians, the diagnosis of sepsis did not require a positive blood culture since it has been shown that many newborns with clinical signs of sepsis had negative cultures in the urine, blood, and cerebrospinal fluid.(Klein 2001)

Culture is a gold standard in diagnosis sepsis although it is positive in only 41.4% cases.(Upadhyay et al., 2006) The organisms causing sepsis differ from region to region, unit to unit, and also time to time within a unit. In Kaiser Permanente Hospital California, the positive culture was only 35.5%.(Escobar et al., 2000)

Isolation of bacteria is the standard and most specific method use to diagnose neonatal sepsis. Important procedures are needed to increase the sensitivity and specificity. These are not always feasible in a very tiny infant.(Buttery 2002)

Culture may be negative because of insufficient bacteria in the sample (due to timing of sampling or degree of bacterial infection), absence of bacteria in the sample (which could be due to maternal intrapartum treatment) although the consequences of infection persist, insufficient specimen quantity, or because another disease might be present.(Escobar 2005)

Laboratory based surveillance will under estimate the prevalence and the burden of BSI. The blood cultures are frequently drawn from patients pretreated with broad-spectrum antibiotics or are not taken at all. In addition, smaller volumes of blood are routinely taken in smaller children, decreasing the sensitivity of the cultures.(See 2005)

For the standard procedure, the minimum blood volume is not known, but generally, 0.75 to 1.0 mL is recommended.(Edwards and Baker, 2003) With computer assisted automated blood culture systems, virtually all cultures containing clinically significant gram positive and negative organisms are positive by 24 to 36 hours of incubation, and cultures containing coagulase-negative *staphylococci* are positive by 48 hours of incubation.(Edwards & Baker, 2003)

Kumar et al (2001) found that among 404 positive blood culture from tertiary neonatal unit, 86% were positive at 36 hours, 96% at 48 hours, and 98.5% by 60 hours. The negative predictive value of a negative blood culture was 97% at 36 hours and 99% at 48 hours.(Kumar et al., 2001)

2.9. TREATMENTS

It should always be remembered that sepsis is treatable and preventable. Improved prevention and treatment could have a substantial effect on survival and quality of life in all children.(Watson and Carcillo, 2005)

The important thing in treatment is : limit use of broad spectrum antibiotics which are potent selectors of antibiotic resistant bacteria. Treat with the most specific antimicrobial agent available based on antibiotic susceptibility testing.(Schelonka, 2007) The choice of antimicrobial therapy is influenced by the following factors : the likely etiologic agent or agents, susceptibility patterns of isolates from infants in specific NICU, the agent's central nervous system penetration, the drug toxicity in neonates, and the infant's hepatic and renal function.(Edwards and Baker, 2003; Saez-Llorenz and McCracken Jr., 2004)

Empirical therapy for early onset sepsis, unchanged after three decades of use, consists of ampicillin and gentamicin. Once meningitis is excluded, ampicillin and gentamicin are employed. Although some experts advocate substitution of cefotaxime or another third generation cephalosporin for gentamicin, this change is not always recommended because : (1) superior efficacy to ampicillin and gentamicin has not been demonstrated, (2) third generation cephalosporins are not active against *Listeria* and *enterococcus spp*., and (3) the routine use of these agents for empirical therapy of sepsis in NICU is associated with "outbreak" of sepsis due to multiple drug resistant organisms. Antibiotic induced depression of beta lactamase leads to resistance to all third generation cephalosporins. Thus, it must be prescribed selectively, not routinely as part of an initial regimen for suspected neonatal infections.(Klein 2001; Edwards and Baker, 2003)

For suspected late onset sepsis occurring within the nursery for which commensal species and methicillin resistant *S. aureus* are possible pathogens, vancomycin and aminoglycoside are empirical agents of choice.(Edwards and Baker, 2003)

However, although suspected sepsis and antibiotic use is common, quality research is required to specifically address both narrow and broad spectrum antibiotics use for early and late onset neonatal sepsis. Future research also needs to assess cost effectiveness and the impact of antibiotics in different settings such as developed or developing countries and lower gestational age groups.(Mtitimila and Cooke, 2004; Gordon and Jeffery 2005)

The dosage, interval and duration of these antibiotics are listed in Table 10. WHO recommends a broader list in Table 11.

The clinician also has to consider the harms and benefits from treatment. Because uncertainty accompanies this decision process regarding infections, the net result is a low antibiotic treatment threshold.(Fischer 2005) It has been estimated that up to 30 otherwise healthy newborns, and up to ten critically ill infants will be treated for every patient in whom infection can ultimately be confirmed.(Fischer 2005) Escobar et al (2000) found that many infants received unnecessary antibiotics as described in table 12.

Beside antibiotics, some other treatments can also be added such as immunoglobulin intravenous (IGIV), PMN transfusion, recombinant granulocytes, monocytes, and granulocyte / macrophage colony stimulating factors. Of these recombinant human granulocyte colony stimulating factors offers the greatest theoretic promise.(Edwards and Baker, 2003)

2.10. PREVENTION

Prevention of neonatal sepsis mainly is divided into 2 approaches : hospital based and community based. A number of effective neonatal care interventions have been identified by Darmstadt et al (2000) and should be considered as priority measures for inclusion in programs aimed at reducing neonatal infections.(Darmstadt et al., 2000; Zaidi et al., 2005)

Table 10. Empirical Antimicrobial Therapy for Neonatal Bacterial Infections

SEPTICEMIA	ANTIBIOTICS (DOSE/KG, IV)	INTERVAL (HOUR)	EXPECTED DURATION (DAYS)
Early-onset (term infant)	Ampicillin (50 mg) + gentamicin (2.5 mg)	q8-12	7-10
Late onset			
• Term infant readmitted	Ampicillin (50 mg) + gentamicin (2.5 mg)	q8-12 q12	7-10
• Inpatient	Vancomycin (15 mg) + gentamicin (2.5 mg) or amikacin (10 mg)	q8 q8	10-14

Source : Edwards and Baker, 2003

Table 11. Empiric Therapy of Serious Neonatal Infections (WHO)

First Line Therapy in Facility Settings

- Early and late onset sepsis : ampicillin plus gentamicin
- Early onset meningitis : ampicillin plus gentamicin
- Late onset meningitis : ampicillin, gentamicin (or amikacin), and/or cefotaxime
- Suspected staphylococcal sepsis, focal skin, bone, joint infections, (including omphalitis),: methicillin/nafcillin plus gentamicin
- For sepsis of suspected gastrointestinal origin : ampicillin, gentamicin/amikacin, plus clindamycin (or piperacillin)
- Nosocomial infection in setting with multi drug resistant *S. aureus* (MRSA) (also if penicillin resistant *pneumococci*, or *enterococci* are suspected): vancomycin plus gentamicin (and/or ceftazidime, if high prevalence of pseudomonas)

Regimens for Community Based Treatment

Parenteral or oral parenteral combination therapy

- Procaine penicillin once daily intramuscular plus gentamicin OD IM
- Amoxillin twice daily orally plus gentamicin OD IM
- Cotrimoxazole twice daily orally plus gentamicin OD IM
- Ceftriaxone IM (as first dose prior to arrival at hospital)

Oral therapy

- Amoxicillin twice daily
- Cotrimoxazole twice daily
- Cefuroxime (or Cefprozil) twice daily

Source : WHO 2003

Table 12. Infection and Treatment Rates among Newborns Evaluated for Bacterial Infection

GROUP	NO	NUMBER INFECTED (%)	NUMBER TREATED (%)
Asymptomatic	1273	12 (0.9)	199 (15.6)
Symptomatic, not critically ill	1329	31 (2.3)	508 (38.2)
Critically ill	183	19 (10.4)	143 (78.4)

Source : Escobar et al., 2000

Based on other considerations, in developed countries, three general approaches for the prevention of neonatal septicemia have been suggested : (1) improvement in prenatal care resulting in delivery of infants at term gestation and without maternal risk factors for septicemia, (2) maternal intrapartum antimicrobial chemoprophylaxis (IAP) for prevention of early-onset Group B *Streptococcal* septicemia and (3) maternal immunoprophylaxis providing Ig-G mediated passive

immunity for the infant to prevent early and late onset GBS septicemia, and potentially caused by other etiologic agents.(Edwards and Baker, 2003) It is clearly noted that outcome among infants whose mother received intrapartum antibiotics are better than among those whose mothers did not.(Escobar et al., 2000; Correia 2006)

In United States in the 1990s intrapartum antibiotic prophylaxis was recommended to prevent maternal infant transmission of GBS. Since IAP has been used, early onset GBS declined 70%.(Lukacs et al., 2004)

In general, it is often impossible to distinguish clinically among sepsis, pneumonia, and meningitis, in the neonate because signs of serious illness are variable, often vague, nonspecific and overlap among these diseases in neonates. Thus in setting in which diagnostic testing is limited and resources are scarce, it may be most prudent to group together and manage in similar manner. (Darmstadt et al., 2000)

Many efforts in neonatal in various levels were already tried with promising results.(Mathur, 2005; Baliga et al., 2006; Haws et al., 2007) In the hospital settings, various attempts were successfully implemented as presented in various reports. (Aly et al., 2005; Zaidi et al., 2005; Saiman 2006; Chudleigh et al., 2007) Hand washing, minimizing invasive procedures, skin preparation, antiseptic solution, probiotic containing anaerob bacteria, disinfection, maintenance of equipment, single use items, infrastructure and staffing, surveillance and monitoring, and implementation strict policy are the examples.(Vergnano et al., 2005; Saiman 2006; Srivastava and Shetty, 2007)

2.11. FUTURE DIRECTION

Infection far from being a homogenous condition reflecting simply the presence of pathogens in blood (bacteremia, viremia), is actually a continuum of phases (Figure 2). The difficulty for the clinician is to determine in which phase the patient is at any given moment as the patient may move from one phase to another in either direction imperceptibly.(Haque 2005)

In the future, 2003 consensus conference proposed PIRO system as a template for investigation. This system would stratify patients based on their : Predisposition (e.g. immunosuppressed state, genetic polymorphism, predicting response to sepsis),

Insult infection (e.g. specific pathogen isolated by culture or bacterial DNA, presence of endotoxin), Response (e.g. presence of shock, levels of inflammatory markers, levels of protein C or TNF alpha that might predict response to specific types of treatment), and Organ dysfunction (e.g. organ failure composite score, measures of cellular response to insult such as apoptosis or mitochondrial dysfunction) (Carrigan et al., 2004; Baron et al., 2005; Escobar 2005) PIRO provide a good framework for what should be reported in observational studies.(Escobar 2005)

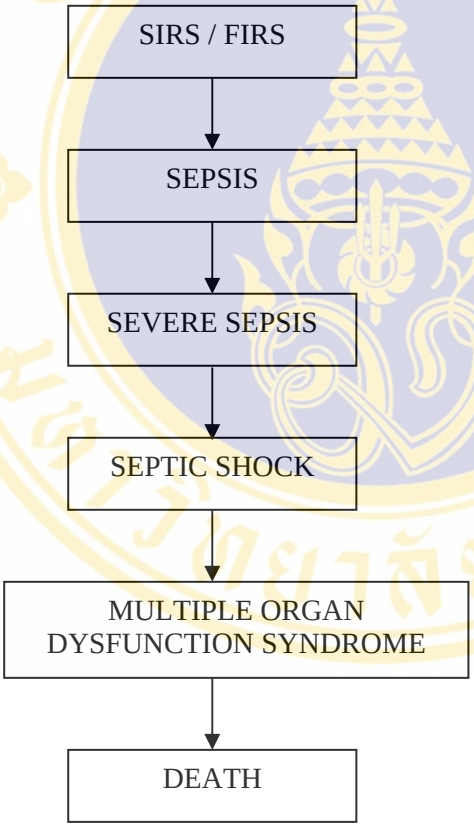

Figure 2. Infection in the Newborn is a Continuum

Case definition is probably best determined first in controlled setting at health care facilities where patients can be monitored closely and outcome determined precisely. Once the model that accurately identifies ill neonates has been developed, it

can be adapted for local culture and field environment and field-tested in community.(Darmstad et al., 2000)

2.12. PREDICTIVE MODEL AND SCORING SYSTEMS

Clinical prediction model (or clinical prediction guide / tool / rules) is a decision-making tool for clinicians that contained 3 or more variables derived from the history, physical examination, and diagnostic test results, and an outcome variable that is either the risk of a disease or event or the need for a treatment or diagnostic test.(Laupacis et al., 1997; Shapiro and Driver, 2004) This predictive model includes simultaneous consideration of several factors in predicting individual patient.(Guyatt 2006) It synthesizes evidence into bedside tools for practice. They are similar to clinical pathway and treatment algorithms in that they are decision support tools that guide or direct the flow of action steps. They differ in that clinical prediction rules are research based and are developed according to strict methodological guidelines.(Shapiro 2005)

In earlier eras prediction model were based on the experienced of respected senior clinicians and took the form of clinical aphorism. More recently, they have been derived from studies involving many hundreds of patients and sophisticated mathematical analysis.(Wasson et al., 1985) It is obvious that clinical experiences provides the clinicians an intuitive sense of which findings on history, physical examinations, and investigations are critical in making an accurate diagnosis or an accurate assessment. However, this intuition may sometimes misleading. Clinicians' experience are memorable but oversimplify complex issues and are subject to many forms of bias. (McGinn et al., 2000)

Prediction model are most useful when the decision being made requires integrating complex data from multiple sources, when the consequences of the decision are potentially risky, or when the decision may result in substantial cost savings without compromising patient care.(McGinn et al., 2000; Shapiro 2005) By making objective the art of diagnosis, predictive model may help clinicians cope with the inevitable uncertainties of clinical practice.(Wasson et al., 1985)

Developing clinical prediction models involves at least 3 important steps, creating or deriving the models, testing or validating (narrow and broad), and

assessing the impact on clinical behavior (narrow and broad impact analysis).(McGinn et al., 2000; Reilly and Evans, 2006) These are listed in Table 13.

Table 13. Developing and Evaluating Clinical Prediction Rules

LEVEL OF EVIDENCE	DEFINITIONS AND STANDARDS OF EVALUATION	IMPLICATIONS FOR CLINICIANS
Level 1 : Derivation of prediction rules	Identification of predictors using multivariate model: blinded assessment of outcomes	Needs validation and further evaluation before using clinically in actual patient care
Level 2 : Narrow validation of prediction rules	Verification of predictors when tested prospectively in 1 setting; blinded assessment of outcomes	Needs validation in varied settings; may use predictions cautiously in patients similar to sample studied
Level 3 : Broad validation of prediction rules	Verification of predictive model in varied settings with wide spectrum of patients and physicians	Needs impact analysis; may use predictions with confidence in their accuracy
Level 4 : Narrow impact analysis of prediction rule used as decision rule	Prospective demonstration in 1 setting that use of prediction rule improves physicians' decisions (quality or cost-effectiveness of patient care)	May use cautiously to inform decisions in settings similar to that studied
Level 5 : Broad impact analysis of prediction rules used as decision rule	Prospective demonstration in varied settings that use prediction rule improves physicians' decision for wide spectrum of patients	May use in varied settings with confidence that its use will benefit patient care quality or effectiveness

Modified from : Reilly and Evans, 2006

In developing clinical prediction models, clinicians begin with a list of potential predictors (can be items from history, physical examination, and laboratory tests) of the outcome of interest. Both the outcome and predictor variables should be chosen through a careful process of thoughtful reflection and attention to methodological detail. Statistical analysis then reveals which predictors are most powerful and which predictors can be omitted from the rules without loss of predictive power. Each analytic method has advantages and limitations.(McGinn et

al., 2000; Shapiro 2006) Developers should describe in detail their choices for each step, justifying the decisions, and accounting for the limitations inherent in the work as they discuss the results of their derivation studies.(Shapiro 2006)

Most prediction rules provide diagnostic or prognostic probabilities, typically using a score or risk stratification algorithm.(Reilly and Evans, 2006). Some others retain the original regression equation from the statistical analysis.

By using scores, clinicians may quantify the morbidity of a neonate. Regarding severity of illness, scoring systems are also used to explain in terms of case mix differences the wide variations in mortality or it may be the estimated probability of a specific outcome (this can be tackled by using an illness severity score). Scoring system involve using appropriately weighted demographic, physiological, and clinical data collected on the infant to calculate a score that quantifies its morbidity.(Dorling et al., 2005) In changing the equation into scoring system, a relative score or point to each predictor in the rule should be derived from the corresponding regression coefficient instead of the odd ratio.(Moons et al., 2002) The items of some neonatal severity scoring systems for case mix purposes and for the specific neonatal sepsis cases are listed on Appendix D.

Clinical prediction model have proliferated in recent years, although very few have undergone formal impact analysis, the standard of evidence to assess their impact on patient care.(Reilly and Evans, 2006)

There are several types of clinical prediction models related with neonatal sepsis. Some examples of them are listed in Table 14.

The predictive model for neonatal sepsis can be based on :

1. Time of neonatal sepsis : early or late.
2. General and specific. Some rules are developed only for neonatal sepsis. The others are general rules. They were used to predict many serious bacterial infections in neonate, either local or systemic.
3. Age category. There were rules for limited age (0-28 days) and also a wider one (for example, 0-90 days).
4. Components. Some rules were consisted of a complete set of history-clinical condition-laboratory test meanwhile some others had limited components only.

5. Validation (limited validation process and widely validated).

Table 14. Some Examples of Clinical Prediction Models for Neonatal Sepsis

NO	SOURCE	CHARACTERISTICS
1	Phillip and Hewitt, 1980	Early
2	St Geme et al., 1984	Early
3	Rodwell et al., 1988	Early, validated
4	Mautone et al., 1989	Early
5	Rodwell et al., 1993	Early
6	Escobar et al., 1994	Early, for discontinuation antibiotic
7	Singh et al., 1994	Early
8	Berger et al., 1995	Early
9	Escobar et al., 1999	Early, to rule out sepsis
10	Phillip, 1982	Late
11	Mahieu et al., 2000	Late, widely validated
12	Mahieu et al., 2002	Late, modification from previous model
13	Singh et al, 2003	Late, clinical only, validated
14	Okascharoen et al., 2005	Late, validated
15	Harrel et al., 1998	General, 0-60 days
16	Bachur and Harper, 2001	General, 0-90 days
17	Bang et al., 2005	Community based
18	Bozaykut et al., 2008	Specific for sepsis of ventilated neonates

CHAPTER III
MATERIALS AND METHODS

3.1. STUDY DESIGN

This was a case control study. All data were compiled from medical records only.

3.2. STUDY SITE

The study was conducted in Queen Sirikit National Institute of Child Health, Bangkok (QSNICH). This hospital has 3 wards for neonatal patients, namely ward 9, ward 10, and NICU.

Ward 9 has 30 beds, 13 nurses for every shift, at least 3 residents, 1 senior staff, and occasionally 1 neonatology fellow. Ward 10 has 30 beds, 9 nurses, and the same amount of medical doctors as ward 9. The NICU has only 8 beds, but the medical personnel is the same as ward 10, except for the residents who are only 2.

Criteria for admitting patients to ward 9 or 10 based are mainly the body weight of the patients. Currently 2000 grams is used as a cutoff (if the body weight is less than 2000 grams, the neonates are admitted to ward 9).

3.3. STUDY PERIOD

The study was performed from October 2007 to March 2008

3.4. DATA SOURCE

Medical record of neonatal patients discharged from QSNICH during the period of 1 October 2004 until 30 September 2007.

3.5. POPULATION AND SAMPLE

3.5.1. Study Population

Neonatal patients admitted in QSNICH (3 sites)

3.5.2. Inclusion Criteria for Sepsis Group

a) Age ≤ 28 days on admission
b) The final diagnosis was sepsis (either main diagnosis or additional)
c) At least the culture result / PCR / staining / antigen-antibody detection was positive for bacterial pathogen
d) The medical record was available

3. 5.3. Exclusion Criteria For Sepsis Group

a) Severe congenital malformation (e.g. severe congenital heart disease, gastroschizis, severe congenital hydrocephalus, and any congenital malformations which are not compatible with life)
b) Admitted for less than 6 hours in hospital
c) Undergone surgery before the diagnosis of sepsis
d) The culture result was viral or fungal infection

3.5.4. Inclusion Criteria for Control Group

a) Age ≤ 28 days on admission
b) The final diagnosis was non-sepsis condition
c) The culture result / PCR / staining / antigen-antibody detection was negative
d) Medical record was available
e) Admitted during the range of 20 days before or after the comparing sepsis patient, except for NICU where the time range is expanded to the same year .
f) Hospitalized in the same ward with the comparing case.
g) Had the same category of age with the comparing case (≤7 days or >7 days) on the day of data taken.

3.5.5. Exclusion Criteria for Control Group

a) Severe congenital malformation (e.g. severe congenital heart disease, gastroschizis, severe congenital hydrocephalus, and any congenital malformations which are not compatible with life)

b) Admitted for less than 6 hours in hospital
c) Undergone surgery before the day when the data was taken
d) Proven bacterial infection

3.5.6. Sample Size Calculation :

Using PS Software, for case control study, independent groups, with :

Alpha = 0.05, Power = 80%,

Probability of exposure in controls = 7% (data from QSNICH 2006 : total neonatal patients 1200, sepsis 298, culture positive 65)

OR = 3, Case : Control = 1: 3

→ Patients to be recruited = 80 : 240

3.6. VARIABLES IN THE ANALYSIS :

3.6.1. Dependent Variable

Dependent variable was proven sepsis.

3.6.2. Independent Variables

Independent variables consisted of :

1. Risk factors (basic / demographic data, maternal history (antepartum, intrapartum, and postpartum))
2. Clinical manifestations
3. Laboratory findings
4. Treatments modalities

The list of independent variables and more detailed explanations were written in the Appendix E.

3.7. DATA ANALYSIS

a) Descriptive
b) Univariate analysis : comparing 2 groups with t-test, Mann Whitney U, or Chi Square test as appropriate
c) Multivariate analysis : multiple logistic regression
d) Diagnostic : Sensitivity, Specificity, PPV, NPV, LR, ROC Curve

Most test used p value < 0.05, two-tailed

Analysis were divided into early and late onset sepsis. Other subgroups analysis (e.g. between preterm and term, and survivor and non-survivor) were also performed.

3.8. STATISTICAL SOFTWARE

SPSS Version 11.5 (SPSS Inc., Chicago, IL) and PS-Power and Sample Size Calculation Software Version 2.1.31 (Vanderbilt, Nashville, TN)

3.9. STUDY PROCEDURE

3.9.1. Data Collection

List of neonatal patients was obtained. The data from medical record in QSNICH were compiled based on the fiscal calendar from October to September. Therefore, the data for this study were taken from the list from October 2004 – September 2007. This list was then divided into three groups : (a) sepsis with positive culture or PCR/stain/latex/antigen-antibody test for bacteria, (b) clinical sepsis, but without positive specific results as mentioned in group (a), and (c) non sepsis. Patients in group (b) were not included in this study. Identification of group (a) was done by finding ICD code of P360 to P368, meanwhile the ICD code for the group (b) was P369.

Then the “sepsis group” was selected. These were all patients with the diagnosis of sepsis (main or additional) with positive culture result or PCR/stain/latex/antigen-antibody tests. Since the number of sepsis group should be more than 80 patients, the original plan to recruit all patients in only 1 fiscal year was expanded to 3 years. To increase the number of subjects in sepsis group, the data from culture result records in neonatal ward was also searched.

All medical records of the sepsis group were checked to ensure the fulfillment of inclusion criteria. Neonates with item listed on the exclusion criteria were not recruited. There were 97 patients with neonatal sepsis recruited, including 45 early neonatal sepsis and 52 late neonatal sepsis patients.

After that, data from medical records of the sepsis group were transferred to case record forms. The dependent variable was proven sepsis. The independent

variables consisted of 4 groups which were risk factors, clinical conditions, laboratory findings, and treatment modalities. For clinical and laboratory examinations, the data used were the worst result (could be highest or lowest) in the range of 24 hours before or after the diagnosis of sepsis. If such data were not available, the most recent previous data were chosen. The name, address, and hospital number of the patients were not recorded in the case record form. The hospital numbers were written in the master log file only.

After obtaining all data for the sepsis group, the patients were divided according to (a) classification of early and late onset sepsis (cutoff 0-7 and >7 days), (b) date of admission, and (c) the ward / site in hospital.

Based on that division and using the master medical record list, the control group was selected. This group will be matched to the sepsis group based on : (a) date of admission (in the range of 20 days before or after the cases) and (b) hospitalized in the same ward (9, 10, or NICU) with the comparing sepsis patient, and (c) age (in the 2 categories namely 1-7 days and more than 7 days). The amount of control : sepsis patients was 3 : 1.

All medical records of the patients in control group were checked to ensure the fulfillment of inclusion criteria. Neonates with item listed in the exclusion criteria were not recruited.

Data from medical record of the patients in control group were transferred to the case record form. For control group of early onset sepsis, the clinical and laboratory examinations data used were the worst in the first 7 days of age. For control group of late onset sepsis the data used were the worst after 7 days of age. For this purpose, the records of clinical conditions and the laboratory results were observed day by day. All patients in the control group did not have diagnosis of sepsis before the data were taken.

All data from case record from were transferred to SPSS data base. Rechecking the data was done every time after 1 form was completely transferred.

3.9.2. Data Management

After all raw data were transferred, cleaning process was done. Illogical numbers (negative number, number outside the range, and blank item) were

rechecked. The original case record form was explored again if necessary. Then some variables were added based on the raw data (such as length of hospital stay before sepsis, modified qualitative variables, and lowest mean arterial pressure). For “yes or no” variables, some cut off values are shown in Appendix F.

3.9.3. Statistical Analysis

The analytical process was begun with evaluation of missing data. Variables with missing data >20% (especially for the sepsis group) were excluded. For the remaining variables, the missing data were replaced. For the sepsis group imputation method was used. While for the control group, the mean of normal value (based on the literature) were chosen. The list of normal value is in Appendix F.

Second, the descriptive analysis was done by finding the frequency distributions, mean $\pm$ SD and median (and range). Categorical variables data were described as number and percentage. Mean and SD were used to describe continuous data if data was normally distributed otherwise median and range was used.

Third, the data were split into 2 categories as planned, the early onset sepsis group and late onset sepsis group. The univariate tests were done. This step compared 2 groups : sepsis and non-sepsis, for each category. The tests used for comparison were t-test, Mann Whitney U, and chi square, depending on the type of the data. Variables with $p > 0.1$ were excluded. The variables with $p \leq 0.1$ were proceed to the next step.

Fourth, for the remaining variables, selection processes were done, based on the clinical consideration, colinearities and similarities, and experience from other researches. Fifth, the multivariate analysis by multiple logistic regression with “enter” method were used for both groups. Since there were many variables, several possibilities were tried. Even after the last result, few variables were tried to reenter the model. The final decision was made based on the number of variables, easiness of usage, clinical judgment, performances, and results from several other studies. The results of these processes were 1 equation for each group.

Sixth, the equations (= models) were tested to find the sensitivity, specificity, PPV, NPV,LR, and the ROC curve on the certain cutoff values. Seventh, for practical purposes, the equations were transformed to score systems. These score

systems were made based on the coefficients of each variable in the equation. Some proposed score systems (vary in the process of rounding the coefficients) were tried for each group and the best results were selected based on the ROC curve. The sensitivity and specificity of each score were also provided.

Finally, the validation step. For each group, from the same sample, a sub-group was chosen randomly by using command “select cases” in the SPSS program. The amount of the sub-group was approximately 35% of the original sample. Both the equation and the score system were validated. This process was repeated twice for each group. The descriptive analysis, the ROC and the Hosmer Lemeshow test were done for the validation step.

3.10. RESEARCH FUND :

Funding for this study was from The Faculty of Tropical Medicine, Mahidol University

3.11. ETHICS COMMITTEE APPROVAL :

Ethical approval of this study was obtained from 2 Ethical Committees, The Faculty of Tropical Medicine, Mahidol University, and Queen Sirikit National Institute of Child Health, both in November 2007.

3.12. SIGNIFICANCE OF RESEARCH :

a) Early diagnostic method for sepsis using risk factors, clinical manifestations, and laboratory results will enable physicians to give early treatment, to avoid unnecessary antibiotics, and to prevent further severe morbidity and mortality.
b) Data about clinical and laboratory diagnosis, risk factors, and treatments can be used for further researches, future planning, and evaluation tools.

CHAPTER IV
RESULTS

4.1. MEDICAL RECORDS SEARCHING

During the study, 550 medical records in the Medical Record Unit QSNICH were explored and at the end, 388 patients were included. There were 97 and 291 patients for cases and controls, respectively. The diagram of the medical records searching is shown in Figure 3.

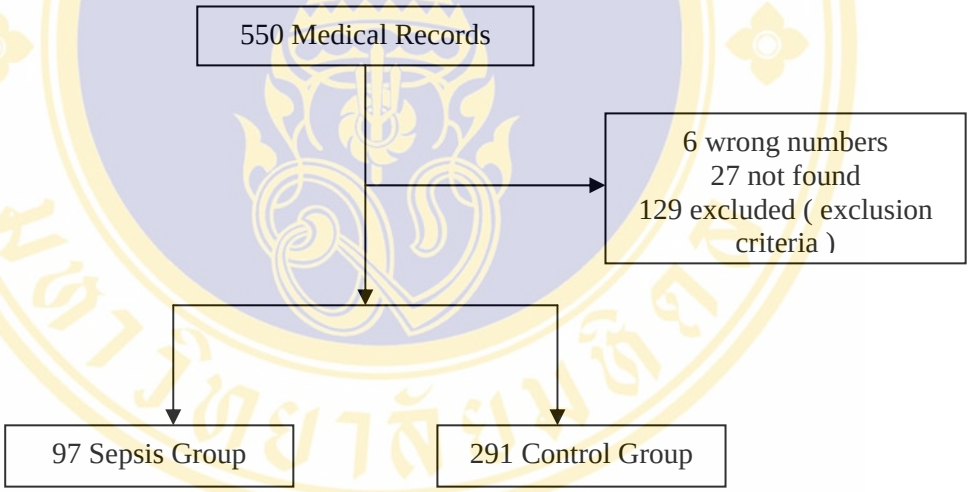

Figure 3. The Medical Records Searching Process

The reason for excluding 129 patients were : clinical sepsis, discharged before the age of 7 days (for the late group), fungal infection, underwent surgery, severe congenital malformations (hydrops fetalis, severe complex congenital heart disease), suspected bacterial infection (for the control patients), and wrong age category.

4.2. BASELINE CHARACTERISTICS

4.2.1. Patient Characteristics

For 3 fiscal years, there were 3557 neonatal patients admitted in QSNICH. The ward distribution of the patients and those who were studied were listed in Table 15. This study used 11% of total neonatal patients. Some baseline characteristics of all neonates and neonates in the study were listed in Table 16 - 18. Most of the neonatal patients in QSNICH were male, had body weight of 2500-4000 grams, and admitted on the first 24 hours of life. Total incidence of all neonatal sepsis patients was 2.7%.

Table 15. Ward Distribution of Neonatal Patients 2004-2007

WARD	2004-2005		2005-2006		2006-2007		SUBTOTAL	
	ALL	USED	ALL	USED	ALL	USED	ALL	USED* n (%)
9	283	72	279	68	228	40	790	180 (22.8)
10	905	88	853	60	879	32	2637	180 (6.8)
NICU	51	8	47	16	32	4	130	28 (21.5)
SUB TOTAL	1239	168	1179	144	1139	76	3557	388 (11%)

Note : USED = used in the study
* = n (% of total patients in that ward)

Table 16. Some Baseline Characteristics of Total Neonatal Patients (3 Fiscal Years)

DATA	WARD 9	WARD 10	NICU	TOTAL
Sex				
• Male	398	1513	72	1983
• Female	392	1124	58	1574
Body Weight				
• ≤ 1000 g	99	7	23	129
• 1001 – 1500	263	9	28	300
• 1501 – 2499	410	512	28	950
• 2500 – 4000	14	1945	49	2008
• 4000 <	4	164	2	170
Age on admission				
• First 24 hours	513	550	75	1138
• > 24 - 72 hours	103	348	22	473
• > 72 hours	78	568	8	654

Table 17. Some Baseline Characteristics of Neonatal Patients in the Study in Case and Control Group

	EARLY SEPSIS		LATE SEPSIS	
	CASE	CONTROL	CASE	CONTROL
• Median age at sepsis / date of data taken for control (days)	4 (1-7)	1 (1-8)	15 (8-28)	10 (8-28)
• Median length of hospital stay before sepsis / date of data taken for control (days)	1 (0-6)	0 (0-7)	11 (0-27)	7 (0-25)
• Median gestational age (weeks)	38 (25-41)	37 (25-42)	32 (26-41)	35 (26-42)
• Mean (SEM) of mother age (years)	26.99 (0.50)	27.18 (1.11)	25.77 (0.98)	26.99 (0.46)
• Maximum space from previous child (years)	23	10	20	10
• Maximum length of hospital stay (days)	141	228	94	114

Table 18. Incidence of Early and Late Onset Neonatal Patients Based on the Body Weight

BODY WEIGHT (Grams)	TOTAL PATIENTS (n)	SEPSIS PATIENTS (n)			INCIDENCE (%)		
		EARLY	LATE	TOTAL SEPSIS	EARLY	LATE	TOTAL SEPSIS
0-1000	129	7	6	13	5.4	4.7	10.1
1001-1500	300	7	18	25	2.3	6	8.3
1501-2499	950	10	11	21	1.1	1.2	2.2
2500-4000	2008	21	16	37	1.1	8	1.8
>4000	170	0	1	1	0	0.6	0.6

4.2.2. Culture Results and Distribution of Microorganisms

The culture method used in QSNICH is BacTec (Becton Dickinson Microbiological System, Maryland).

There were 97 neonatal patients who showed positive culture results from the blood (hemoculture). Fifty seven of them (58.8%) had more than 1 positive samples. Three patients also had positive gram stain from CSF, and 1 had positive latex agglutination test from CSF. All of those gram stain and latex agglutination test results were comparable with the hemoculture.

Among the control group, 2 patients had positive hemoculture for CONS and 1 had positive ELISA test for dengue infection. However, the data from these 2 patients with CONS were taken before the culture procedures.

The distribution of microorganisms from hemoculture were listed on the Table 19.

Table 19. Distribution of Microorganisms by Early and Late Sepsis

EARLY SEPSIS (N (%))		LATE SEPSIS (N (%))	
Acinetobacter spp (including 2 *A. calcoaceticus baumanii*)	11(24.4)	*Klebsiella pneumoniae* (including 6 ESBL)	16 (30.8)
Staphylococcus aureus (including 1 MRSA)	8 (17.8)	CONS	7 (13.5)
Klebsiella pneumoniae (including 1 ESBL, and 1 double infection with *Serratia*)	6 (13.3)	*Enterobacter* spp.	6 (11.5)
		Staphylococcus aureus (including 1 MRSA)	4 (7.7)
		Escherichia coli	4 (7.7)
		Pseudomonas spp.	4 (7.7)
Escherichia coli	5 (11.1)	*Acinetobacter spp*	3 (5.8)
CONS	3 (6.7)	Group B *Streptococcus*	2 (3.9)
Enterococcus spp.	3 (6.7)	*Enterococcus*	1 (1.9)
Group B *Streptococcus*	3 (6.7)	*Citrobacter* spp.	1 (1.9)
Enterobacter spp.	2 (4.4)	*Serratia*	1 (1.9)
Citrobacter spp.	1 (2.2)	*Streptococcus agalactiae*	1 (1.9)
Haemophilus influenzae	1 (2.2)	*Streptococcus viridans*	1 (1.9)
Pseudomonas spp.	1 (2.2)	*Streptococcus pneumoniae*	1 (1.9)
Serratia (plus 1 double infection with *K. pneumoniae*)	1 (2.2)		
TOTAL	**45 (100)**		**52 (100)**

4.2.3. Diagnosis in Non-Sepsis Group

Among 291 control patients, the major diagnoses were hyperbilirubinemia (72% neonates in the early sepsis group and 77% from the late sepsis group had this diagnosis, either as a main or additional one), asphyxia, and respiratory problem such as respiratory distress syndrome, apnea of prematurity, or other respiratory distress conditions.

4.3. MISSING DATA

Variables with missing data $\geq$20%, especially for the sepsis group, were excluded. High rate of missing data reflected that these variables were not important or not routinely done. Thirty three and 25 variables were excluded for early and late sepsis, respectively.

Table 20. Variables with Missing Data More than 20%

EARLY SEPSIS	LATE SEPSIS
1. Respiratory pattern	1. Respiratory pattern
2. Band form	2. Band form
3. I:T ratio	3. I:T ratio
4. Erythrocyte sedimentation rate	4. Erythrocyte sedimentation rate
5. Arterial pO_2	5. Arterial pO_2
6. Abnormal calcium	6. Abnormal calcium
7. AST	7. AST
8. Abnormal AST	8. Abnormal AST
9. ALT	9. ALT
10. Abnormal ALT	10. Abnormal ALT
11. Abnormal LFT	11. Abnormal LFT
12. CRP1	12. Direct bilirubin
13. CRP2	13. Abnormal direct bilirubin (> 0.2 mg/dl)
14. CSF turbidity	14. Abnormal direct bilirubin (> 2 mg/dl)
15. CSF pandy	15. Total bilirubin
16. CSF pandy – qualitative	16. Abnormal total bilirubin
17. CSF color	17. CRP1
18. CSF color – qualitative	18. CRP2
19. CSF opening pressure	19. CSF opening pressure
20. CSF closing pressure	20. CSF closing pressure
21. CSF WBC	21. Urine protein
22. CSF WBC – qualitative	22. Urine glucose
23. CSF glucose	23. Urine sediment WBC
24. CSF glucose - qualitative	24. Urine sediment RBC
25. Percentage CSF glucose: blood glucose	25. Urine cast
26. Percentage CSF glucose: blood glucose – qualitative	
27. CSF protein	
28. CSF protein - qualitative	
29. Urine protein	
30. Urine glucose	
31. Urine sediment WBC	
32. Urine sediment RBC	
33. Urine cast	

4.4. COMPARISON OF OUTCOME, AGE AND LENGTH OF HOSPITAL STAY BETWEEN CASE AND CONTROL (Table 21)

In neonatal sepsis, most of the patients (53.3%) developed sepsis during the age of 4-7 days for early sepsis and 15-28 days for late sepsis. These are difference from the control group ($p<0.001$).

In early neonatal sepsis, most of the patients (51.2%) developed sepsis within 24 hours of hospitalization. In contrast, most of the patients (63.4%) developed late neonatal sepsis after hospitalization for 7 days.

The patients who had sepsis had significantly higher mortality and longer hospitalization comparing to the control group.

4.5. COMPARISON OF RISK FACTORS BETWEEN CASE AND CONTROL

The comparison of risk factors between case and control were listed in Table 22 – 26. Odds ratio regarding the risk factors for sepsis were listed in Table 27.

In the early and late sepsis group, more than 50% of neonatal sepsis patients were born from the high risk pregnancies, comparing with only 35% in the control. Most of their mothers were in the 15-30 years group of age, and had occupations as labor or did not work at all, and lived at the slum area. The majority education of them were elementary and high school.

In the late sepsis group, 51.9% mothers received steroid injection before birth which had protective effect for the neonates. Premature rupture of the membranes in the sepsis group were not significantly different from the control group for both early and late neonatal sepsis. Only 6 mothers had chorioamnionitis in this study. Among complication of pregnancy, preeclampsia was the most common (5 and 9 cases for early and late sepsis group, respectively). Majority of all neonates had good apgar score either for the first or fifth minutes.

Table 21. Comparison of Outcome, Age, Length of Hospital Stay, Sex, and Referral Source between Case and Control

	EARLY SEPSIS			LATE SEPSIS		
	CASE (n (%))	**CONTROL (n (%))**	**p**	**CASE (n (%))**	**CONTROL (n (%))**	**p**
Outcome			0.140			**0.001**
• Alive	37(82.2)	122(90.4)		44(84.6)	153(98.1)	
• Death	8(17.8)	13(9.6)		8(15.4)	3(1.9)	
Age on admission			0.561			0.264
• 0-24 hours	28(62.2)	80(59.3)				
• >24-72 hours	11(24.4)	27(20)				
• >72 hours	6(13.4)	28(20.7)				
Age on sepsis (/ date of data taken for control)			**<0.001**			**<0.001**
• 0-24 hours	5(11.1)	68(50.4)		-	-	
• 2-3 days	16(35.6)	36(26.7)		-	-	
• 4-7 days	24(53.3)	31(22.9)		-	-	
• 8-14 days	-	-		22(42.3)	120(76.9)	
• 15-28 days	-	-		30(57.7)	36(23.1)	
Length of hospital stay before sepsis (/ date of data taken for control)			**<0.001**			**<0.001**
• 0-24 hours	23(51.2)	129(95.6)		13(25)	62(39.7)	
• 2-3 days	11(24.4)	4(3)		1(2)	6(3.9)	
• 4-7 days	11(24.4)	2(1.4)		5(9.6)	43(27.6)	
• 8-14 days	0	0		14(26.9)	39(24.9)	
• >14 days	-	-		19(36.5)	6(3.9)	
Total length of hospital stay			**<0.001**			**<0.001**
• 0-24 hours	0	8(5.9)		1(2)	3(1.9)	
• 2-3 days	2(4.4)	19(14.1)		0	23(14.7)	
• 4-7 days	3(6.7)	25(18.5)		0	20(12.8)	
• >7 days	40(88.9)	83(61.5)		-	-	
• 8-14 days	-	-		5(9.6)	31(19.9)	
• 15-30 days	-	-		14(26.9)	41(26.3)	
• >30 days	-	-		32(61.5)	38(24.4)	
Sex			0.120			0.470
• Male	20(44.4)	78(57.8)		26(50)	87(55.8)	
• Female	25(55.6)	57(42.2)		26(50)	69(44.2)	
Referred from			0.344			0.606
• Rajvithi hospital	30(66.7)	85(63)		25(48.1)	75(48.1)	
• Other hospital	12(26.7)	27(20)		15(28.8)	36(23.1)	
• Others	3(6.6)	22(16.3)		12(23.1)	45(28.8)	
• Missing	-	1		-	-	

Table 22. Comparison of Risk Factors between Case and Control Group – Antepartum History (I)

	EARLY SEPSIS			LATE SEPSIS		
	CASE (n (%))	**CONTROL (n (%))**	**p**	**CASE (n (%))**	**CONTROL (n (%))**	**p**
ANTEPARTUM HISTORY						
Frequency of ANC			0.334			**0.004**
• 0	2(4.5)	12(8.9)		9(17.3)	8(5.1)	
• 1-4	15(33.3)	23(17)		17(32.7)	34(21.8)	
• >4	28(62.2)	100(74.1)		26(50)	114(73.1)	
Race			0.333			0.327
• Thai	45(100)	130(96.3)		50(96.2)	152(97.4)	
• Non-Thai	0	5(3.7)		1(1.9)	4(2.6)	
• Mixed	0	0		1(1.9)	0	
Mother's age			**0.014**			0.085
• 15-20 years	11(24.4)	19(14.1)		18(34.6)	25(16)	
• 21-30 years	22(48.9)	86(63.7)		19(36.5)	88(56.4)	
• 31-35 years	7(15.6)	18(13.3)		11(21.2)	30(19.2)	
• >35 years	5(11.1)	12(8.9)		4(7.7)	13(8.4)	
Smoking			0.439			0.438
• Yes	1(2.2)	1(0.7)		1(1.9)	1(0.6)	
• No	44(97.8)	134(99.3)		51(98.1)	155(99.4)	
Alcohol			1.00			C
• Yes	0	1(0.7)		0	0	
• No	45(100)	134(99.3)		52(100)	156(100)	
Genital disease			1.00			1.00
• Yes	0	2(1.5)		0	1(0.6)	
• No	45(100)	132(97.8)		52(100)	154(98.7)	
• Missing	-	1		-	1	
Chronic disease of the mother			0.385			0.218
• Yes	12(26.7)	26(19.3)		6(11.5)	17(10.9)	
• No	33(73.3)	109(80.7)		46(88.5)	138(88.5)	
• Missing	-	-		-	1	
High risk pregnancy			**0.009**			**0.002**
• Yes	26(57.8)	48(35.6)		30(57.7)	52(33.3)	
• No	19(42.2)	87(64.4)		22(42.8)	104(66.7)	
Long term drug			0.076			1.00
• Yes	6(13.3)	6(4.4)		2(3.8)	6(3.8)	
• No	39(86.7)	129(95.6)		50(96.2)	150(96.2)	
Education			**0.005**			0.123
• None	0	5(3.7)		1(1.9)	0	
• Elementary	19(42.2)	82(60.7)		24(46.2)	95(60.9)	
• High school	20(44.4)	38(28.2)		22(42.3)	51(32.7)	
• University	6(13.4)	9(6.7)		4(9.6)	10(6.4)	
• Missing	-	1(0.7)		1	-	

Table 23. Comparison of Risk Factors between Case and Control Group – Antepartum History (II)

	EARLY SEPSIS			LATE SEPSIS		
	CASE (n (%))	**CONTROL (n (%))**	**p**	**CASE (n (%))**	**CONTROL (n (%))**	**p**
ANTEPARTUM HISTORY - (II)						
Occupation			**0.024**			0.459
• None	17(37.8)	76(56.3)		29(55.8)	76(49)	
• Office	5(11.1)	9(6.7)		4(9.6)	13(8.3)	
• Shop	1(2.2)	6(4.5)		1(1.9)	11(7.1)	
• Labor	18(40)	42(31.1)		15(28.9)	49(31.4)	
• Government	3(6.7)	1(0.7)		2(3.8)	6(3.8)	
• Others	1(2.2)	0		1(1.9)	0	
• Missing	-	1		-	1	
Environment			**<0.001**			**0.013**
• Slum	29(64.4)	117(86.7)		39(75)	125(80.1)	
• Factory	5(11.1)	0		4(7.7)	0	
• Market	5(11.1)	7(5.2)		4(7.7)	17(10.9)	
• Others	6(13.4)	10(7.4)		5(9.6)	13(8.3)	
• Missing	-	1		-	1	
Number of pregnancy			0.280			0.225
• 1	22(48.9)	78(57.8)		24(47.1)	98(62.8)	
• 2-3	20(44.4)	51(37.8)		24(46.2)	50(32.1)	
• >3	3(6.7)	6(4.4)		3(6.7)	8(5.1)	

For the early sepsis group, the highest significant odds ratio for risk factors between sepsis and non-sepsis-patients were found on length of hospital stay before sepsis, long term drug usage, high risk pregnancy, and icterus after birth. For the late sepsis group, the highest odds ratio were found on length of hospital stay, intracranial hemorrhage, high risk pregnancies, and resuscitation.

Table 24. Comparison of Risk Factors between Case and Control Group – Intrapartum History (I)

	EARLY SEPSIS			LATE SEPSIS		
	CASE (n (%))	CONTROL (n (%))	p	CASE (n (%))	CONTROL (n (%))	p
INTRAPARTUM HISTORY - (I)						
Mode of delivery			0.140			0.561
• Spontaneous	30(66.7)	64(47.4)		28(53.9)	85(54.5)	
• Caesarian section	12(26.6)	59(43.7)		22(42.3)	66(42.3)	
• Others	3(6.7)	12(8.9)		2(3.8)	5(3.2)	
Premature rupture of the membrane >8 hours			1.00			0.105
• Yes	5(11.1)	15(11.1)		8(15.3)	12(7.7)	
• No	40(88.9)	120(88.9)		43(82.7)	144(92.3)	
• Missing	-	-		1	-	
Chorioamnionitis			0.439			1.00
• Yes	1(2.2)	1(0.7)		1(1.9)	3(1.9)	
• No	44(97.8)	134(99.3)		47(90.4)	153(98.1)	
• Missing	-	-		4	-	
Smelly amniotic fluid			C			0.555
• Yes	0	0		1(1.9)	2(1.3)	
• No	45(100)	135(100)		47(90.4)	154(98.7)	
• Missing	-	-		4	-	
Color of amniotic fluid			1.00			0.222
• Normal	41(91.1)	123(91.1)		45(86.5)	151(96.8)	
• Turbid	0	0		1(1.9)	1(0.6)	
• Green	4(8.9)	12(8.9)		3(5.8)	4	
• Missing	-	-		3	-	
Fever of the mother			0.166			0.597
• Yes	3(6.7)	3(2.2)		2(3.9)	3(1.9)	
• No	42(93.3)	132(97.8)		48(92.3)	153(98.1)	
• Missing	-	-		2	-	
Complication of pregnancy			**0.02**			0.056
• Yes	13(28.9)	22(16.3)		19(36.5)	32(20.5)	
• None	32(71.1)	113(83.7)		33(63.5)	124(79.5)	
Location of birth			0.732			0.575
• Hospital	43(95.6)	130(96.3)		52(100)	153(98.1)	
• Non hospital	2(4.4)	5(3.7)		0	3(1.9)	
Birth helper			0.6			C
• Medical personnel	43(95.6)	132(97.8)		52(100)	156(100)	
• Others	2(4.4)	3(2.2)		0	0	

Table 25. Comparison of Risk Factors between Case and Control Group – Intrapartum History (II)

	EARLY SEPSIS			LATE SEPSIS		
	CASE (n (%))	CON TROL (n (%))	p	CASE (n (%))	CON TROL (n (%))	p
INTRAPARTUM HISTORY – (II)						
Steroid injection before birth			0.299			**0.003**
• Yes	16(35.6)	37(27.4)		27(51.9)	46(29.5)	
• No	29(64.4)	98(72.6)		25(48.1)	110(70.5)	
Antibiotic injection before birth			0.816			0.279
• Yes	19(42.2)	61(45.2)		26(51)	66(42.3)	
• No	25(56.8)	74(54.8)		25(48.1)	90(57.7)	
• Missing	1	-		1	-	
Singleton			0.823			0.619
• Yes	42(93.3)	121(89.6)		48(92.3)	134(85.9)	
• Twins	3(6.7)	13(9.6)		4(7.7)	20(12.8)	
• Triplets	0	1(0.8)		0	2(1.3)	
Birth weight			0.596			0.240
• 1-1000 grams	7(15.6)	5(3.6)		6(11.5)	4(2.6)	
• 1001-1500 grams	7(15.6)	16(11.9)		18(34.6)	33(21.2)	
• 1501-2499 grams	10(22.1)	45(33.3)		11(21.2)	6(3.9)	
• 2500-4000 grams	21(46.7)	68(50.4)		16(30.8)	53(34)	
• >4000 grams	0	1(0.8)		1(1.9)	0	
Gestational age			0.786			**0.041**
• 20-27 weeks	4(8.9)	4(3)		2(3.8)	3(2)	
• 28-36 weeks	18(40)	60(44.3)		34(65.4)	95(60.9)	
• 37-41 weeks	23(51.1)	70(51.9)		16(30.8)	57(36.5)	
• >41 weeks	0	1(0.8)		0	1(0.6)	
Apgar 1			0.072			**0.023**
• 0-3	4(8.9)	12(8.9)		8(15.4)	8(5.1)	
• 4-6	10(22.1)	15(11.1)		6(11.5)	19(12.2)	
• 7-10	26(57.8)	105(77.8)		34(65.3)	126(80.8)	
• Missing	5	3		4	3	
Apgar 5			0.709			**0.014**
• 0-3	2(4.4)	5(3.6)		1(1.9)	0	
• 4-6	2(4.4)	6(4.4)		8(15.4)	10(6.4)	
• 7-10	36(80)	121(89.6)		39(81.3)	143(91.7)	
• Missing	5	3		4	3	
Lubchenko			0.104			0.075
• AGA	40(88.9)	129(95.6)		45(86.5)	146(93.6)	
• SGA	5(11.1)	4(3)		4(7.7)	10(6.4)	
• LGA	0	2(1.4)		2(3.8)	0	
• Missing	-	-		1	-	

Table 26. Comparison of Risk Factors between Case and Control Group – Intrapartum History (III) and Postpartum History

	EARLY SEPSIS			LATE SEPSIS		
	CASE (n (%))	CONTROL (n (%))	p	CASE (n (%))	CONTROL (n (%))	p
INTRAPARTUM HISTORY – (III)						
Intracranial hemorrhage			1.00			**0.019**
• Yes	3(6.7)	9(6.7)		9(17.3)	9(5.8)	
• No	42(93.3)	126(93.3)		43(82.7)	147(94.2)	
Umbilical cord			1.00			0.250
• Fresh	44(97.8)	133(98.5)		51(98.7)	156(100)	
• No	1(2.2)	2(1.5)		1(1.3)	0	
Resuscitation			0.719			**0.026**
• Yes	17(37.8)	47(34.8)		26(50)	52(33.3)	
• No	28(62.2)	88(65.2)		26(50)	104(66.7)	
Main mode of resuscitation			0.267			**0.002**
• Intubation	14(82.4)	29(61.7)		21(80.8)	26(50)	
• Oxygen mask	3(17.6)	18(38.3)		5(19.2)	26(50)	
Length of resuscitation			0.407			**0.033**
• 1-20 seconds	1(5.9)	1(2.1)		3(11.5)	2(3.9)	
• 21-60 seconds	6(33.3)	36(76.6)		20(76.9)	48(92.3)	
• 61-300 seconds	5(29.4)	3(6.4)		1(3.9)	1(1.9)	
• >300 seconds	5(29.4)	7(14.9)		2(7.7)	1(1.9)	
POSTPARTUM HISTORY						
Taking care postnatally by medical personnel	45(100)	135(100)	C	52(100)	156(100)	C
Icterus after birth			**0.028**			0.770
• Yes	10(22.2)	13(9.6)		5(9.6)	12(7.7)	
• No	35(77.8)	122(90.4)		47(90.4)	144(92.3)	
Seizure			0.061			1.00
• Yes	2(4.4)	0		1(1.9)	2(1.3)	
• No	43(95.6)	135(100)		51(98.1)	154(98.7)	
Fever of the baby			**0.049**			**<0.001**
• Yes	3(6.7)	1(0.7)		7(13.5)	0	
• No	42(93.3)	134(99.3)		45(86.5)	156(100)	
Breastfed			0.214			0.205
• Yes	7(15.6)	33(24.4)		14(26.9)	57(36.5)	
• No	38(84.4)	102(75.6)		38(73.1)	99(63.5)	

Table 27. Odds Ratio for Risk Factors between Sepsis and Non-Sepsis Patients

VARIABLES	EARLY SEPSIS		LATE SEPSIS	
	OR	95% CI	OR	95% CI
DEMOGRAPHIC				
• Sex (male : female)	1.711	0.867 - 3.376	1.261	0.672 - 2.364
• Length of hospital stay before sepsis (<4 : ≥4 for early) and (<7:≥7 for late)	**21.515**	4.553 – 101.671	**4.284**	2.209 – 8.308
ANTEPARTUM HISTORY				
• Race (Thai : non-Thai)	-	-	1.520	0.270 - 8.550
• Smoking (Yes : No)	3.045	0.187 - 49.472	3.039	0.187 - 49.472
• Alcohol (Yes : No)	-	-	-	-
• Genital disease (Yes : No)	0.256	0.022 - 2.042	-	-
• Chronic disease of mother (Yes : No)	1.517	0.739 - 3.116	1.000	0.374 - 2.671
• High risk pregnancy (Yes : No)	**2.840**	1.246 - 4.938	**2.727**	1.434 - 5.188
• Long term drug user (Yes : No)	**3.308**	1.009 - 10.839	1.000	0.196 - 5.114
INTRAPARTUM HISTORY				
• Mode of delivery I (Caesarian section : non-operative)	**0.468**	0.223 - 0.985	1.000	0.53 - 1.888
• Mode of delivery II (Non-spontaneous : spontaneous)	**0.451**	0.222 - 0.913	1.026	0.547 - 1.926
• Premature rupture of membrane (Yes : No)	1.000	0.342 - 2.926	2.233	0.857 - 5.815
• Chorioamnionitis (Yes : No)	3.045	0.187 - 49.175	1.085	0.110 - 10.680
• Smelly amniotic fluid (Yes : No)	-	-	1.638	0.145 - 18.742
• Fever of mother (Yes : No)	3.143	0.611 - 16.162	2.125	0.345 -13.093
• Steroid injection before birth (Yes : No)	0.684	0.334 - 1.403	**0.387**	0.203 - 0.737
• Antibiotics before birth (Yes : No)	1.085	0.509-1.964	0.756	0.39-1.467
• Prematurity (20-36 weeks: 37≤)	1.000	0.546 - 2.154	0.705	0.374 - 1.330
• Intracranial hemorrhage (Yes : No)	1.000	0.259 - 3.867	**3.419**	1.277 - 9.149
• Umbilical cord (Not Fresh : Fresh)	1.511	0.134 - 17.074	-	-
• Resuscitation (Yes : No)	1.137	0.565 - 2.287	**2.060**	1.084 - 3.916
POSTPARTUM HISTORY				
• Icterus after birth (Yes : No)	**2.681**	1.084 - 6.635	1.277	0.428 - 3.812
• Seizure (Yes : No)	-	-	1.510	0.134 - 17.000
• Fever of the baby (Yes : No)	9.571	0.970 - 94.475	-	-
• Breastfed (Yes : No)	1.756	0.716 - 4.305	1.563	0.781 - 3.128

4.6. COMPARISON OF CLINICAL CONDITION BETWEEN CASE AND CONTROL

Table 28. Comparison of Clinical Condition between Sepsis and Non-Sepsis Patients

	EARLY SEPSIS			LATE SEPSIS		
	CASE (n (%))	**CONTROL (n (%))**	**p**	**CASE (n (%))**	**CONTROL (n (%))**	**p**
Lethargy			**<0.001**			**<0.001**
• Yes	32(71.1)	27(20)		35(67.3)	18(11.5)	
• No	13(28.9)	108(80)		17(32.7)	138(88.5)	
Poorfeeding			**<0.001**			**<0.001**
• Yes	30(66.7)	23(17)		36(69.2)	17(10.9)	
• No	15(33.3)	112(83)		16(30.8)	139(89.1)	
Cyanosis			0.299			0.554
• Yes	1(2.2)	11(8.1)		5(9.6)	11(7.1)	
• No	44(97.8)	124(91.9)		47(90.4)	145(92.9)	
Icterus on admission			0.255			**0.002**
• Yes	16(35.6)	36(26.7)		5(9.6)	49(31.4)	
• No	29(64.4)	99(73.3)		47(90.4)	107(68.6)	
Hypotension			0.160			1.00
• Yes	31(68.9)	77(57)		50(96.2)	151(96.8)	
• No	14(31.1)	58(43)		2(3.8)	5(3.2)	
Abnormality of heart rate			**0.003**			**<0.001**
• Yes	7(15.6)	3(2.2)		18(34.6)	2(1.3)	
• No	38(84.4)	132(97.8)		34(65.4)	154(98.7)	
Abnormality of temperature			**0.029**			**<0.001**
• Yes	8(17.8)	8(5.9)		17(32.7)	3(1.9)	
• No	37(82.2)	127(94.1)		35(67.3)	153(98.1)	
Mechanical ventilation			0.086			**<0.001**
• Yes	24(53.3)	46(34)		35(67.3)	23(14.7)	
• No	21(46.7)	89(66)		17(32.7)	133(85.3)	
Respiratory insufficiency			**0.016**			**<0.001**
• Yes	31(68.9)	65(48.1)		42(80.8)	43(27.6)	
• No	14(31.1)	70(51.9)		10(19.2)	113(72.4)	
Seizure in hospital			0.369			1.00
• Yes	3(6.7)	4(3)		1(1.9)	5(3.2)	
• No	42(93.3)	131(97)		51(98.1)	151(96.8)	
Capilarry refill			0.432			C
• Normal	44(97.8)	134(99.3)		49(100)	156(100)	
• Not normal	1(2.2)	1(0.7)		0	0	
Oliguria			0.338			0.062
• Yes	0	0		0	0	
• No	45(100)	135/100		52(100)	156(100)	
Abnormal oxygen saturation			**<0.001**			**<0.001**
• Yes	15(33.3)	12(8.9)		23(44.2)	11(7)	
• No	23(51.1)	123(91.1)		25(52.1)	145(93)	
• Missing	7	-		4	-	

Table 28 and 29 compared the clinical conditions between case and control. Most of these variables showed significant differences between cases and controls for both groups, early and late sepsis. Odds ratio regarding clinical conditions for sepsis were listed in Table 29. The highest odd ratio for early sepsis was apnea episode, meanwhile for late sepsis was abnormal heart rate.

Table 29. Odds Ratio for Clinical Conditions between Sepsis and Non-Sepsis Patients

VARIABLES	EARLY SEPSIS		LATE SEPSIS	
	OR	**95% CI**	**OR**	**95% CI**
• Lethargy (Yes : No)	**9.846**	4.557 - 21.272	**15.874**	7.385 - 33.737
• Poorfeeding (Yes : No)	**9.739**	4.531 - 20.933	**18.397**	8.477 - 39.927
• Cyanosis (Yes : No)	0.256	0.032 - 2.042	1.402	0.463 - 4.243
• Icterus in hospital (Yes : No)	1.517	0.739 - 3.116	**0.232**	0.087 - 0.620
• Abnormal heart rate (Yes : No)	**8.105**	1.999 – 32.862	**40.765**	9.029 – 184.041
• Abnormal temperature (Yes : No)	**3.432**	1.206 – 9.771	**24.771**	6.880 – 89.195
• Apnea episode (Yes : No)	**14.235**	3.761 – 53.883	**17.000**	4.616 – 62.608
• Respiratory insufficiency (Yes : No)	**2.385**	1.166 – 4.878	**11.037**	5.090 – 23.931
• Hypoxemia (Yes : No)	**9.804**	4.265 – 22.540	**14.236**	6.273 – 32.309

4.7.COMPARISON OF LABORATORY DATA BETWEEN CASE AND CONTROL GROUP

The comparison of laboratory data between case and control were listed on Table 30 - 34. Odds ratio of laboratory data for sepsis were listed in Table 35. The highest odds ratio for early sepsis was leucopenia, meanwhile for late sepsis was abnormal CSF glucose.

Table 30. Comparison of Laboratory Data (Complete Blood Count, Blood Gas Analysis, and Electrolytes) between Sepsis and Non-Sepsis Patients

	EARLY SEPSIS			LATE SEPSIS		
	CASE (n (%))	CONTROL (n (%))	p	CASE (n (%))	CONTROL (n (%))	p
CBC						
Anemia			**<0.001**			**<0.001**
• Yes	20(44.4)	24(17.8)		28(53.9)	29(18.6)	
• No	25(55.6)	111(82.2)		24(46.1)	127(81.4)	
Leucopenia			**<0.001**			**0.004**
• Yes	13(28.9)	4(3)		4(7.7)	0	
• No	32(71.1)	131(97)		48(92.3)	156(100)	
Abnormal leucocytes–Manroe			**0.002**			**<0.001**
• Yes	28(62.2)	49(36.3)		19(36.5)	5(3.2)	
• No	17(37.8)	86(63.7)		33(63.5)	151(96.8)	
Thrombocytopenia			**<0.001**			**<0.001**
• Yes	23(51.1)	16(11.8)		23(44.2)	12(7.7)	
• No	22(48.9)	119(88.2)		29(55.8)	144(92.3)	
Abnormality of neutrophil-Manroe			0.922			**<0.001**
• Yes	28(62.2)	49(36.3)		24(46.1)	14(9)	
• No	17(37.8)	86(63.7)		28(53.9)	142(91)	
BLOOD GAS ANALYSIS						
Abnormal arterial blood pH			**<0.001**			**<0.001**
• Yes	25(55.6)	42(31.1)		36(69.2)	22(14.1)	
• No	14(31.1)	93(68.9)		13(25)	134(85.9)	
• Missing	6	-		3	-	
Abnormal base excess			**<0.001**			**<0.001**
• Yes	17(37.8)	25(18.8)		20(38.5)	11(7)	
• No	22(48.9)	110(81.2)		29(55.8)	145(93)	
• Missing	6	-		3	-	
ELECTROLYTES						
Abnormal potassium			**0.002**			**<0.001**
• Yes	25(55.6)	41(30.4)		32(61.5)	44(28.2)	
• No	15(33.3)	94(69.6)		19(36.5)	112(71.8)	
• Missing	5	-		1	-	
Abnormal sodium			0.079			**0.001**
• Yes	11(24.4)	18(13.3)		12(23.1)	10(6.4)	
• No	29(64.4)	117(86.7)		39(75)	146(93.6)	
• Missing	5	-		1	-	

Table 31. Comparison of Laboratory Data (Blood Sugar, RFT, and Bilirubin) between Sepsis and Non-Sepsis Patients

	EARLY GROUP			LATE GROUP		
	CASE (n (%))	CONTROL (n (%))	p	CASE (n (%))	CONTROL (n (%))	p
BLOOD SUGAR						
Hypoglycemia			1.00			0.062
• Yes	1(2.2)	2(1.5)		2(3.9)	0	
• No	32(71.1)	133(98.5)		46(88.5)	156(100)	
• Missing	12	-		4	-	
Hyperglycemia			**0.002**			**0.001**
• Yes	26(57.8)	80(59.3)		36(69.2)	77(49.4)	
• No	7(15.6)	55(40.7)		12(23.1)	79(50.6)	
• Missing	12	-		4	-	
Hyperglycemia (>180 mg/dl)			0.439			0.250
• Yes	1(2.2)	80(59.3)		1(1.9)	0	
• No	32(71.1)	55(40.7)		47(90.4)	156(100)	
• Missing	12	-		4	-	
Abnormal glucose (<40;>180 mg/dl)			**0.001**			**0.015**
• Yes	27(60)	82(60.7)		38(73.1)	77(49.4)	
• No	6(13.3)	53(39.3)		10(19.2)	79(50.6)	
• Missing	12	-		4	-	
RFT						
Abnormal BUN			**<0.001**			**<0.001**
• Yes	10(22.2)	7(5.2)		10(19.2)	6(3.8)	
• No	26(72.2)	128(94.8)		33(63.5)	150(96.2)	
• Missing	9	-		9	-	
Abnormal creatinine			0.109			C
• Yes	4(8.9)	4(3)		0	0	
• No	32(71.1)	131(97)		43(100)	156(100)	
• Missing	9	-		9	-	
Abnormal RFT			**<0.001**			**<0.001**
• Yes	10(22.2)	10(7.4)		10(19.2)	6(3.8)	
• No	26(57.8)	125(92.6)		33(63.5)	150(96.2)	
• Missing	9	-		9	-	
BILIRUBIN						
Abnormal direct bilirubin			0.061			-
• Yes	2(4.4)	0		-	-	
• No	35(77.8)	135(100)		-	-	
• Missing	8	-		-	-	
Abnormal total bilirubin			0.372			-
• Yes	10(22.2)	52(38.5)		-	-	
• No	27(60)	83(61.5)		-	-	
• Missing	8	-		-	-	

Table 32. Comparison of Laboratory Data (Cerebrospinal Fluid Findings) between Sepsis and Non-Sepsis Patients

	EARLY GROUP			LATE GROUP		
	CASE (n (%))	CONTROL (n (%))	p	CASE (n (%))	CONTROL (n (%))	p
Abnormal CSF leucocytes			-			**<0.001**
• Yes	-	-		17(32.7)	5(3.2)	
• No	-	-		35(67.3)	151(96.8)	
Abnormal CSF glucose			-			**<0.001**
• Yes	-	-		17(32.7)	3(1.9)	
• No	-	-		35(67.3)	153(98.1)	
Abnormal CSF protein			-			**<0.001**
• Yes	-	-		14(26.9)	5(3.2)	
• No	-	-		38(73.1)	151(96.8)	

Table 33. Comparison of Some Clinical Condition and Laboratory Data (Blood Pressure and CBC) between Sepsis and Non-Sepsis Patients

VARIABLES	EARLY SEPSIS (Mean (SEM))			LATE SEPSIS (Mean (SEM))		
	CASE	CONTROL	p	CASE	CONTROL	p
CLINICAL						
- Mean lowest systolic (mmHg)	54.489 (1.488)	57.089 (0.701)	0.082	58.5 (1.704)	60.494 (0.746)	0.221
- Mean lowest diastolic (mmHg)	30.844 (0.981)	33.163 (0.590)	**0.049**	33.539 (1.607)	35.699 (0.630)	0.093
- Mean lowest mean arterial pressure (mmHg)	42.667 (1.171)	45.126 (0.597)	**0.048**	46.019 (1.375)	48.096 (0.638)	0.128
- Mean oxygen saturation (%)	86.316 (2.239)	93.366 (0.571)	**<0.001**	87.174 (0.593	95.417 (0.483)	**<0.001**
CBC						
- Mean hemoglobin (g/dl)	13.833 (0.426)	15.737 (0.195)	**<0.001**	12.389 (0.315)	14.859 (0.170)	**<0.001**
- Mean lowest thrombocytes (/cmm)	177391.11 (24842.3)	239092.54 (7681.95)	**0.022**	219819.27 (26832.81)	316673.11 (11260.49)	**0.001**
- Mean absolute neutrophils (/cmm)	8991.989 (897.109)	8901.604 (491.473)	0.922	10755.19 (1202.33)	5764.610 (273.026)	**<0.001**

Table 34. Comparison of Some Clinical Condition and Laboratory Data (BGA, Blood Sugar, RFT, and Bilirubin) between Sepsis and Non-Sepsis Patients

VARIABLES	EARLY SEPSIS (Mean (SEM))			LATE SEPSIS (Mean (SEM))		
	CASE	CONTROL	p	CASE	CONTROL	p
BLOOD GAS ANALYSIS						
- Mean pH	7.295 (0.020)	7.352 (0.014)	**<0.001**	7.295 (0.023)	7.388 (0.005)	**<0.001**
BLOOD SUGAR, RFT, AND BILIRUBIN						
- Mean blood sugar (mg/dl)	97.667 (7.452)	86.667 (2.754)	**<0.001**	92.75 (6.376)	74.801 (2.011)	**0.005**
- Mean BUN (mg/dl)	17.177 (2.50)	15.33 (2.550)	**0.001**	16.513 (1.874)	9.222 (0.430)	**<0.001**
- Mean creatinine (mg/dl)	0.739 (0.078)	0.794 (0.037)	0.718	0.488 (0.028)	0.547 (0.010)	**0.025**
- Mean direct bilirubin (mg/dl)	1.411 (0.522)	0.665 (0.017)	**0.039**	- -	- -	-
- Mean total bilirubin (mg/dl)	10.064 (1.442)	11.906 (0.553)	0.829	- -	- -	-

Table 35. Odds Ratio for Laboratory Data between Sepsis and Non-Sepsis Patients

VARIABLES	EARLY SEPSIS		LATE SEPSIS	
	OR	95% CI	OR	95% CI
• Anemia (Yes : No)	**3.7**	1.744 – 7.717	**5.109**	2.593 – 10.067
• Leucopenia (Yes : No)	**13.305**	4.066 – 43.534	-	-
• Abnormal leucocytes (Manroe's) (Yes : No)	**2.891**	1.439 – 5.806	**17.388**	6.056 – 49.921
• Thrombocytopenia (Yes:No)	**7.776**	3.552 – 17.023	**9.517**	4.260 – 21.264
• Abnormal pH (Yes : No)	**4.903**	2.366 – 10.162	**18.273**	8.437 – 39.577
• Abnormal base excess (Yes : No)	**4.6**	2.221 – 9.528	**10.455**	4.597 – 23.778
• Abnormal potassium (Yes : No)	**2.866**	1.433 – 5.731	**4.073**	2.108 – 7.869
• Abnormal sodium (Yes : No)	2.103	0.906 – 4.879	**4.380**	1.764 – 10.872
• Hypoglycemia (Yes : No)	-	-	-	-
• Abnormal glucose (Yes : No)	**4.201**	1.644 – 10.609	-	-
• Abnormal BUN (Yes : No)	**9.143**	3.427 – 24.392	**10.135**	3.681 – 27.095
• Abnormal CSF WBC (Y:N)	-	-	**14.669**	5.068 – 42.456
• Abnormal CSF glucose (Y:N)	-	-	**24.771**	6.880 – 89.195
• Abnormal CSF protein (Y:N)	-	-	**11.126**	3.774 – 32.806

4.8.COMPARISON OF TREATMENT MODALITIES BETWEEN CASE AND CONTROL GROUP

The comparison of treatment modalities between case and control group were listed in Table 36 and 37. All data of vascular catheter and TPN were taken before the diagnosis of sepsis. The highest odds ratio for early and late sepsis was central or umbilical catheterization. Immunoglobulin, blood component, and exchange transfusion data were presented for treatment pattern analysis.

Table 36. Comparison of Treatment Modalities between Sepsis and Non-Sepsis Patients

	EARLY SEPSIS			LATE SEPSIS		
	CASE (n (%))	**CONTROL (n (%))**	**p**	**CASE (n (%))**	**CONTROL (n (%))**	**P**
Immunoglobulin			**0.015**			**<0.001**
• Yes	3(6.7)	0		7(13.5)	0	
• No	42(93.3)	135(100)		45(86.5)	156(100)	
Blood component			**<0.001**			**<0.001**
• Yes	31(68.9)	38(28.1)		36(69.2)	42(26.9)	
• No	14(31.1)	97(71.9)		16(30.8)	114(73.1)	
Exchange transfusion			**<0.001**			0.125
• Yes	8(17.8)	1(0.7)		5(9.6)	5(3.2)	
• No	37(82.2)	134(99.3)		47(90.4)	151(96.8)	
Vascular catheter			**0.02**			**0.001**
• Yes	45(100)	110		52(100)	128()	
• No	0	25		0	28	
Central / Umbilical catheter			**<0.001**			**<0.001**
• Yes	26(57.8)	37(27.4)		35(67.3)	37(23.7)	
• No (without catheter, or with venous catheter)	19(42.2)	98(72.6)		17(32.7)	119(76.3)	
Total parenteral nutrition			0.085			**<0.001**
• Yes	28(62.2)	64(47.4)		40(76.9)	57(36.5)	
• No	17(37.8)	71(52.6)		12(23.1)	99(63.5)	

Table 37. Odds Ratio for Treatment Modalities between Sepsis and Non-Sepsis Patients

VARIABLES	EARLY SEPSIS		LATE SEPSIS	
	OR	**95% CI**	**OR**	**95% CI**
• Vascular catheter (Yes : No)	-	-	-	-
• Central / Umbilical catheter (Yes : No)	**3.624**	1.796 – 7.314	**6.622**	3.332 – 13.161
• Total parenteral nutrition (Yes : No)	1.827	0.916 – 3.646	**5.789**	2.810 – 11.927

4.9. VARIABLE FOR MULTIVARIATE ANALYSIS

All the significant results from the univariate tests (53 for early and 68 variables for late sepsis) were reselected (based on colinearities, similarities, and clinical judgment) in order to determine which variables would go to multivariate analysis. The best choices were listed in Table 38. There were 19 and 26 variables for early and late sepsis groups, respectively.

Table 38. Variables for Multivariate Analysis

EARLY SEPSIS	LATE SEPSIS
RISK FACTORS	**RISK FACTORS**
1. Length of hospital stay before sepsis - qualitative	1. Length of hospital stay before sepsis - qualitative
2. Mode of delivery (spontaneous/not)	2. Steroid before birth
3. Apgar 1 – qualitative	3. Gestational age
4. Apgar 5 - qualitative	4. Apgar 1 – qualitative
5. Seizure	5. Apgar 5 - qualitative
6. Fever of the baby (after birth)	6. Intracranial hemorrhage
CLINICAL CONDITION	7. Resuscitation (yes/no)
7. Poorfeeding	8. Length of resuscitation – qualitative
8. Mean arterial pressure	9. Fever of the baby
9. Abnormal heart rate	**CLINICAL CONDITION**
10. Abnormal temperature	10. Poorfeeding
11. Mechanical ventilation	11. Icterus in hospital
12. Respiratory insufficiency	12. Diastolic blood pressure
13. Abnormal oxygen saturation	13. Abnormal heart rate
LABORATORY	14. Abnormal temperature
14. Anemia	15. Mechanical ventilation
15. Leucopenia	16. Respiratory insufficiency
16. Thrombocytopenia	17. Urine production
17. Abnormal arterial blood pH	18. Abnormal oxygen saturation
TREATMENT	**LABORATORY**
18. Central/umbilical catheter	19. Anemia
19. Total parenteral nutrition	20. Abnormal leucocytes
	21. Thrombocytopenia
	22. Absolute neutrophils
	23. Abnormal arterial blood pH
	24. CSF WBC - qualitative
	TREATMENT
	25. Central/umbilical catheter
	26. Total parenteral nutrition

4.10. THE EQUATION AND THE PROBABILITY OF PROVEN SEPSIS

Multiple logistic regression produced results for early and late sepsis that written in 2 boxes. There were 5 variables in the final regression equation for early and 6 variables for late sepsis group. The result of the regression equation was placed in the exponential equation in order to achieve the probability. "Probability" in this equation means "probability to have proven sepsis" and is expressed as percentage. The odds ratio and adjusted odds for all variables in the equations were shown in Table 39 and 40.

The equation for early sepsis consisted of 1 risk factors, 2 clinical conditions, and 2 laboratory data. The equation for late sepsis consisted of 4 clinical conditions and 2 laboratory data.

Table 39. Odds Ratio and Adjusted Odds of Variables Used in The Equation for Early Sepsis

VARIABLES	ODDS RATIO	95% CI		ADJUST ODDS	95% CI	
		LOWER	UPPER		LOWER	UPPER
RISK FACTORS						
- Length of hospital stay >3 days	21.515	4.553	101.671	10.634	1.711	66.097
CLINICAL						
- Poor feeding	9.739	4.531	20.933	5.503	1.362	9.265
- Abnormal oxygen saturation	9.804	4.265	22.540	4.425	2.332	19.022
LABORATORY						
- Thrombocytopenia	7.776	3.552	17.023	4.493	1.683	12.927
- Leucopenia	13.305	4.066	45.534	9.719	1.456	28.999

Table 40. Odds Ratio and Adjusted Odds of Variables Used in The Equation for Late Sepsis

VARIABLES	ODDS RATIO	95% CI		ADJUST ODDS	95% CI	
		LOWER	UPPER		LOWER	UPPER
CLINICAL						
- Poor feeding	18.397	8.477	39.927	10.996	3.201	37.776
- Abnormal heart rate	40.765	9.029	184.041	21.920	2.235	215.020
- Abnormal temperature	24.771	6.880	89.195	54.334	6.997	421.915
- Abnormal oxygen saturation	14.236	6.273	32.309	4.004	1.019	15.729
LABORATORY						
- Abnormal leucocytes	17.388	6.056	49.921	5.967	1.162	30.645
- Abnormal pH	18.273	8.437	39.577	11.924	3.583	39.690

THE FINAL EQUATION AND THE PROBABILITY FOR EARLY SEPSIS

EQUATION :

Y = (2.364 * length of hospital stay before sepsis) + (1.268 * poorfeeding)
+ (1.896 * abnormal oxygen saturation)
+ (1.540 * thrombocytopenia) + (1.872 * leucopenia)
- 2.895

VARIABLES AND CODING :

Length of hospital stay before sepsis
- < 4 days = 0
- 4-7 days = 1

Poorfeeding
- Yes = 1
- No = 0

Abnormal oxygen saturation (< 92%)
- Yes = 1
- No = 0

Thrombocytopenia (<150 000 /cmm)
- Yes= 1
- No = 0

Leucopenia (≤ 5000 / cmm)
- Yes = 1
- No = 0

PROBABILITY :

P = {EXP (Y)} / { 1 + EXP (Y) }

THE FINAL EQUATION AND THE PROBABILITY FOR LATE SEPSIS

EQUATION :

Y = (2.398 * poorfeeding) + (3.087 * abnormal heart rate)
+ (3.995 * abnormal temperature) + (1.387 * abnormal oxygen saturation)
+ (1.786 * abnormal leucocytes)
+ (2.479 * abnormal pH) – 4.328

VARIABLES AND CODING :

Poorfeeding
- Yes = 1
- No = 0

Abnormal heart rate (Normal range : 100 – 180 x/minute)
- Yes = 1
- No = 0

Abnormal temperature (Normal range : 36 – 37.9^{o}C)
- Yes = 1
- No = 0

Abnormal oxygen saturation (< 92%)
- Yes = 1
- No = 0

Abnormal leucocytes → Normal range :

< 7 days of age	: 9000 – 30 000 /cmm
7-14 days of age	: 5000 – 21 000 /cmm
> 14 days of age	: 5000 – 20 000 /cmm

- Yes = 1
- No = 0

Abnormal pH (Normal range : 7.27 – 7.45)
- Yes = 1
- No = 0

PROBABILITY :

P = {EXP (Y)} / { 1 + EXP (Y) }

4.11. THE SCORE

To make the final equation easier for application, 2 scoring systems were also derived (each for early and late sepsis group, respectively). The score were made based on the coefficients of the variables in the final equation. Some possibilities (in rounding the coefficients) for the scores were also tried and the best choice was selected based on the area under the curve of the ROC curve. Both scoring system were presented in the boxes below.

For early sepsis, the score consisted of 5 variables as the probability equation. For late sepsis, the score consisted of 6 variables. The appropriateness between the score and the probability were also presented.

<table>
<tr>
<td>

THE SCORE FOR EARLY SEPSIS

Length of stay before sepsis
- < 4 days = 0
- 4-7 days = 2

Poorfeeding
- Yes = 1
- No = 0

Abnormal oxygen saturation (< 92%)
- Yes = 2
- No = 0

Thrombocytopenia (<150 000 /cmm)
- Yes= 1
- No = 0

Leucopenia (≤ 5000 / cmm)
- Yes = 2
- No = 0

</td>
<td>

Maximum Score is :
8

The score equal with the probability in the range :

- Low :
0 – 1 = 0 – 25%
- Medium :
2 – 3 = 26 – 79%
- High :
4 - 8 = 80 - 100%

</td>
</tr>
<tr>
<td>

THE SCORE FOR LATE SEPSIS

Poorfeeding
- Yes = 2
- No = 0

Abnormal heart rate (100 – 180 x/minute)
- Yes = 3
- No = 0

Abnormal temperature (36 – 37.9°C)
- Yes = 4
- No = 0

Abnormal oxygen saturation (< 92%)
- Yes = 1
- No = 0

Abnormal leucocytes

< 7 days of age : 9000 – 30 000 /cmm
7-14 days of age : 5000 – 21 000 /cmm
> 14 days of age : 5000 – 20 000 /cmm

- Yes = 2
- No = 0

Abnormal pH (7.27 – 7.45)
- Yes = 2
- No = 0

</td>
<td>

Maximum Score is :
14

The score equal with the probability in the range :

- Low :
0 – 2 = 0 – 20%
- Medium :
3 – 4 = 21 – 75%
- High :
5 - 6 = 76 - 95%
- Very High :
7-14 = 96 - 100%

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</table>

In order to make it clearer, the relation between the probability (equation) and the scoring system were presented by the scatter diagram on Figure 4 and 5.

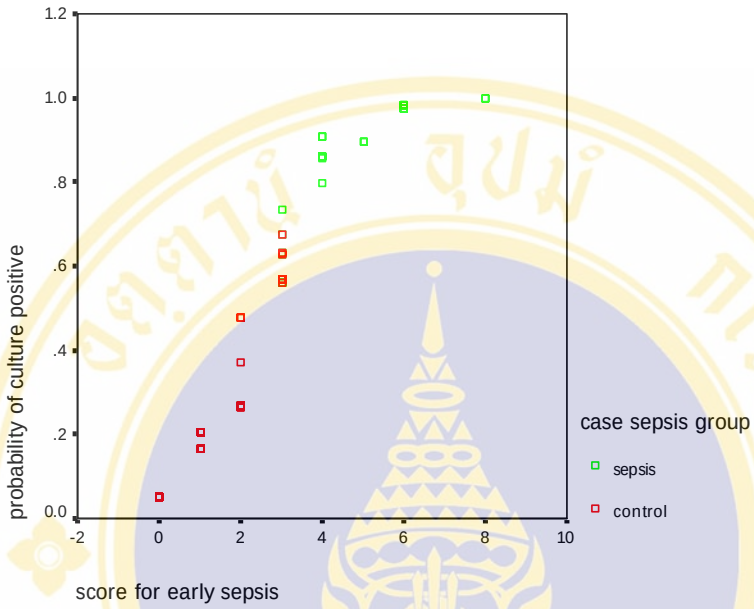


Figure 4. The Score System Reflects The Probability of Proven Sepsis of Early Sepsis

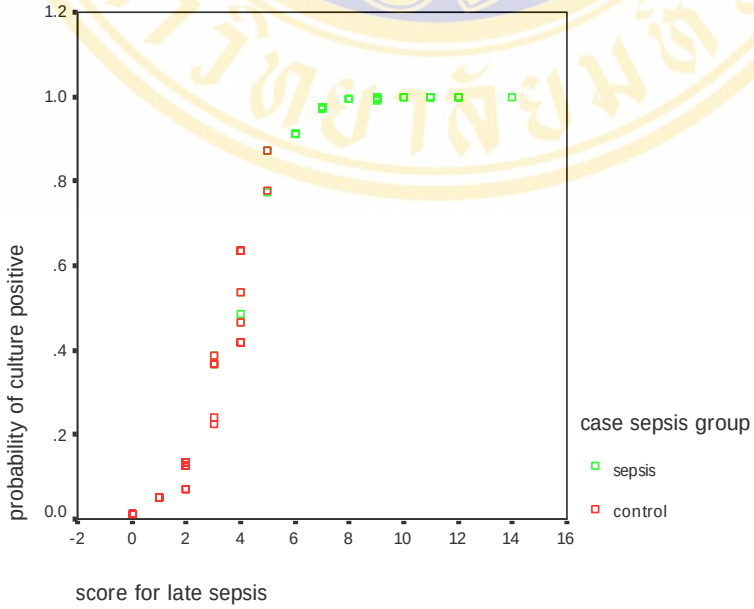


Figure 5. The Score System Reflects The Probability of Proven Sepsis for Late Sepsis

4.12. EVALUATION OF PERFORMANCE

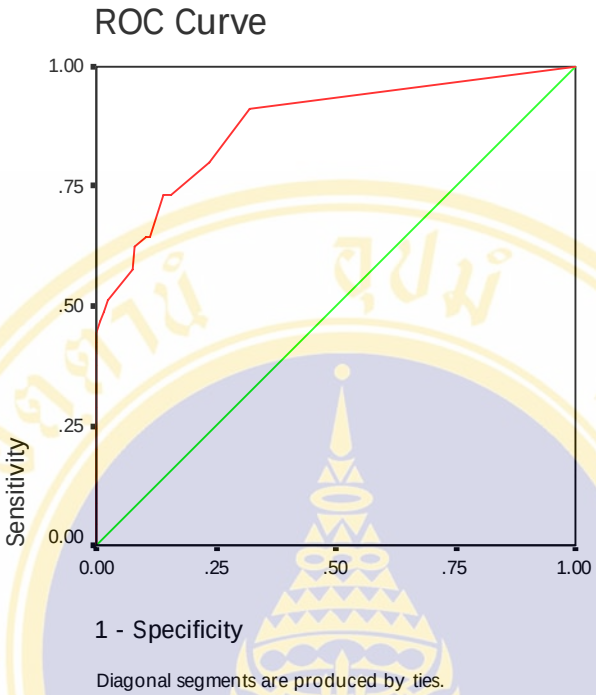
The performance (sensitivity, specificity, PPV, NPV, LR+, LR(-), and ROC curve) of the equation and the scoring system for early and late sepsis group were presented in Table 41 - 44 and Figure 6 – 9.

Table 41. Performance of The Equation for Early Sepsis

PROBABILITY CUTOFF (%)	PROBABILITY	
	SENSITIVITY	SPECIFICITY
0	100	0
10.00	91.1	68.2
20.00	80	76.3
30.00	64.4	88.9
40.00	64.4	89.6
50.00	62.2	91.9
60.00	51.1	97.8
70.00	44.4	100
80.00	40	100
90.00	24.4	100
100.00	0	100

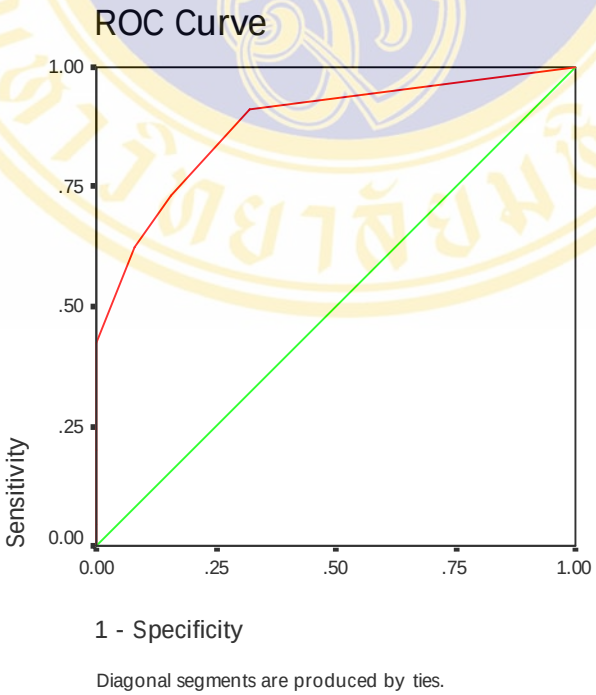
Table 42. Performance of The Score System for Early Sepsis

CUTOFF (TOTAL SCORE)	SCORE SYSTEM					
	SENSITIVITY	SPECIFICITY	PPV	NPV	LR+	LR-
0	91.1	68.2	48.8	95.8	2.87	0.13
1	73.3	84.4	61.1	90.5	4.70	0.32
2	62.2	91.9	71.8	87.9	7.68	0.41
3	42.2	100	100	83.9		0.58
4	24.4	100	100	79.9		0.76
5	20.0	100	100	79.0		0.80
6	4.4	100	100	75.8		0.96
7	4.4	100	100	75.8		1
8	0	100	100	75.0		



AUC 87.7%

Figure 6. ROC of The Equation of Early Sepsis



AUC 87.8%

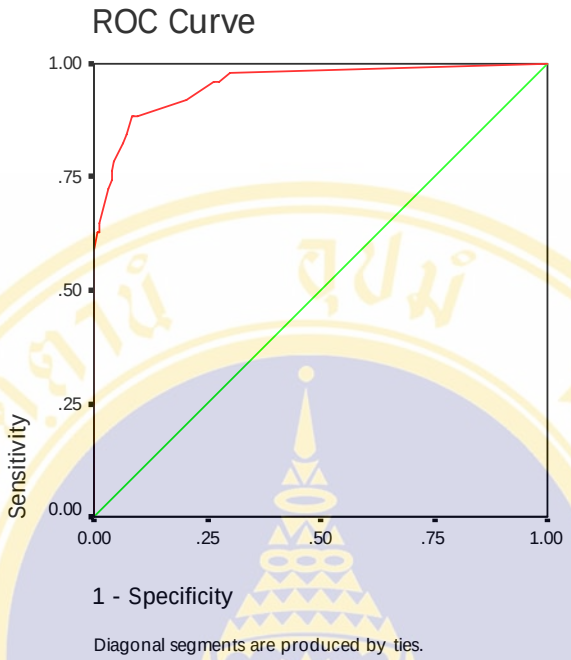
Figure 7. ROC of The Score System of Early Sepsis

Table 43. Performance of The Equation for Late Sepsis

PROBABILITY CUTOFF (%)	EQUATION	
	SENSITIVITY	SPECIFICITY
0	100	0
10.00	94.2	73.7
20.00	86.6	90.4
30.00	86.6	91.7
40.00	80.8	93.6
50.00	73.1	96.2
60.00	71.2	96.8
70.00	63.5	98.7
80.00	61.5	99.4
90.00	57.7	100
100.00	0	100

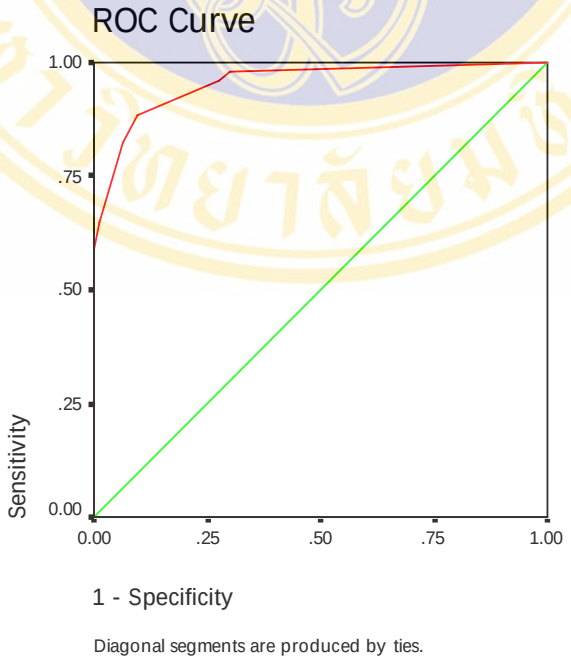
Table 44. Performance of The Scoring System for Late Sepsis

CUTOFF (TOTAL SCORE)	SCORE SYSTEM					
	SENSITIVITY	SPECIFICITY	PPV	NPV	LR+	LR-
0	98.1	69.9	52	99.1	3.26	0.03
1	96.2	72.4	53.8	98.3	3.49	0.05
2	88.5	90.4	75.4	95.9	9.22	0.13
3	82.7	93.6	81.1	94.2	12.92	0.19
4	65.4	98.7	94.4	89.5	50.31	0.35
5	59.6	100	100	88.1		0.40
6	50	100	100	85.7		0.50
7	34.6	100	100	82.1		0.65
8	28.9	100	100	80.8		0.71
9	19.2	100	100	78.8		0.81
10	13.5	100	100	77.6		0.87
11	7.7	100	100	76.5		0.92
12	1.9	100	100	75.4		0.98
13	1.9	100	100	75.4		0.98
14	0	100	100	75		1



AUC 95.6%

Figure 8. ROC of The Equation of Late Sepsis



AUC 95.5%

Figure 9. ROC of The Score System of Late Sepsis

4.13. VALIDATION

Validation process for both equation and score system for early and late sepsis were done using 35% samples randomly taken (computer generated) from the initial data set. The distribution of the samples for all validation groups were shown in Table 45. The AUC of ROC curves of the equations and the scores for all validation groups, either early or late sepsis, were above 80%. All results of the Hosmer-Lemeshow Tests showed p>0.05.

Table 45. Distribution of Validation Groups and Results of Validation Tests

ITEM	EARLY SEPSIS		LATE SEPSIS	
	VALIDATION GROUP 1	VALIDATION GROUP 2	VALIDATION GROUP 1	VALIDATION GROUP 2
1. Total patients	66	56	76	82
2. Culture positive patients	16	17	18	25
3. Total death patients	9	5	1	5
4. Percentage patients from ward 9 : 10 : NICU (%)	36.4 : 49.9 : 13.7	35.7 : 51.8 : 12.5	46.1 : 50 : 3.9	53.7 : 45.1 : 1.2
5. Median length of hospital stay before sepsis (days)	0	0	7	7
6. Median age on admission (days)	1.00	1.00	1.50	1.50
7. Median body weight (grams)	2562.5	2655	2220	1830
8. Median gestational age (weeks)	37	38	36	35
9. AUC of ROC curve for Equation (%)	83.1	86.6	96.6	93.6
10. AUC of ROC curve for Score (%)	82.2	86.2	96.3	93.6
11. Hosmer Lemeshow Tests for Score	p=0.442	p=0.776	p=0.897	p=0.079

4.14. TREATMENTS OF NEONATAL SEPSIS

All sepsis patients (100%) received antibiotics. Only 4 (4.1%) patients receive a single antibiotic. The maximum numbers of antibiotics in 1 patient were 7 items.

Immunoglobulin were given to 10 (10.3%) patients, and blood components to 67 (69.1%) sepsis patients. There were 13 patients (13.4%) received exchange transfusion during hospitalization.

All sepsis patients had at least 1 vascular catheter in the body. It was either umbilical, arterial, or venous catheter. Sixty eight (70.1%) patients received total parenteral nutrition and 62 (63.9%) needed artificial ventilation.

4.15. SEASONAL VARIATION OF NEONATAL SEPSIS

Bar diagram in Figure 10 showed the distribution of the sepsis patients by month. Comparison between early and late sepsis group for the whole year showed p=0.577 (Mann-Whitney U Test). Comparison between sepsis cases in the first (January-June; 37 sepsis cases among 1697 neonatal patients) and second semester (July-December; 60 sepsis cases among 1860 neonatal patients) showed significant difference with p=0.035.

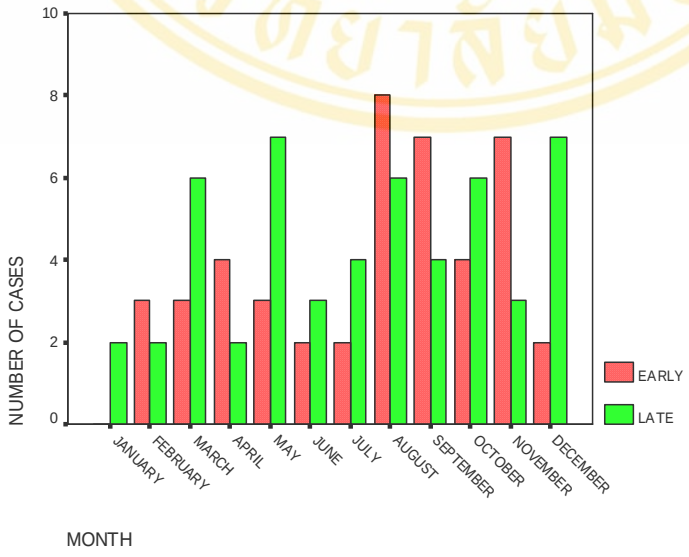


Figure 10. Seasonal Variation of Neonatal Sepsis

CHAPTER V
DISCUSSION

5.1. INCIDENCE

Ninety seven sepsis patients were identified in this study among 3557 neonatal patients during the 3 fiscal years study period. The overall incidence of proven neonatal sepsis in QSNICH was 2.7% (denominator : all neonatal patients in QSNICH). The incidence of proven very early (VEOS), early (EOS) and late onset (LOS) neonatal sepsis were 0.14%, 1.27% and 1.46%, respectively. VEOS patients was 5% (5/97) of all neonatal sepsis patients. Since QSNICH does not have normal neonates in the unit, it is difficult to know the incidence per 1000 live birth. Comparisons among countries were also not easy since many reports used different criteria for the term early and late.(Vergnano et al., 2005)

The incidence of neonatal sepsis in one study in Bahrain was 4.19%.(Bindayna et al., 2006) The incidence of proven LOS in this study was higher than in Ramathibodi Hospital in Bangkok (0.01%). The Ramathibodi study used 3 days as a criteria for late onset sepsis.(Okascharoen et al., 2005)

A large study in Australia and New Zealand found the incidence of late neonatal sepsis episodes of 0.85% (3113/368,765).(Gordon et al., 2006) Yale-New Haven Hospital in USA maintained the longest running single-center longitudinal database of neonatal sepsis since 1928. They found that the percentage of cases of early onset sepsis decreased while the late onset sepsis increased, compared with the previous 10 year study period.(Bizzarro et al., 2005)

The median age of LOS in this study was 15 days. Fanaroff et al., found median age of 17 days in their study.(Fanaroff et al., 1998)

In NICU only, the incidence of all neonatal sepsis in QSNICH was 2.5%. This number is relatively lower than the situation in Chinju, South Korea (6.7% total neonatal sepsis cases for 10 years period study in NICU), and in Taipei, Taiwan (3.01% total neonatal sepsis cases for 10 years period study in NICU).(Jiang et al.,

2004; Park et al., 2007) Two studies from India reported the incidence of 9.5% and 51%, respectively.(Vasudevan et al., 2005; Upadhyay et al., 2006)

Among all neonatal sepsis, the percentage of neonates whose weight less than 2500 grams was 64.1%. The incidence of neonatal sepsis in the patients with body weight less than 2500 grams were definitely higher than in the normal body weight neonates as stated in many other reports. In India, the percentage of LBW in neonatal sepsis was 84.6% and in Korea 12.7%.(Upadhyay et al., 2006; Park et al., 2007) In Taiwan, proportion of VLBW among all neonatal sepsis was 54.4%.(Jiang et al., 2004)

For ELBW, the incidence of EOS was higher than in Norway while for the LOS was lower.(Ronnestad et al, 2005; Ronnestad et al., 2005b)

Based on the gestational age, percentage of preterm neonates among all neonatal sepsis were 48.9%, 69.2%, and 59.8% for EOS, LOS, and total sepsis, respectively. These results were similar to other body weight based reports.

The case fatality in EOS, LOS, and total neonatal sepsis in QSNICH were 17.8%, 15.4%, and 16.5%, respectively. Five of 8 death patients (62.5%) in each category were preterm (and BW < 2500 grams). Total case fatality in the report from Korea was 12%.(Park et al., 2007) In Taiwan the case fatality rate was 16.3%, significantly higher in EOS group, but not significantly higher in VLBW. (Jiang et al., 2004) In India, the rate was 34-38.1%.(Vasudevan et al., 2005; Upadhyay et al., 2006) In Australia, the case fatality rate of LOS was 20.8% and significantly related with body weight and gestational age.(Gordon and Isaacs, 2006) Study from USA revealed the mortality for EOS was decreased 5% annually (1995-1998); assumed to be due to GBS prophylaxis guidelines. While for the LOS, it was increased 5% annually during the same period.(Lukacs et al., 2004)

5.2. MICROORGANISM PATTERN

The percentage of gram negative organisms for EOS and LOS in this study were 62.2% (28/45) and 67.3% (35/52), respectively. *Klebsiella pneumoniae* was the most common microorganism. These data were comparable with other developing countries.(Vergnano et al., 2005; Ghotaslou et al., 2007) In Australia the percentage of gram negative was only 22.5%.(Gordon and Isaacs, 2006)

For early sepsis, the microorganism pattern was different from some reports. *Acinetobacter spp.* (including *A. calcoaceticus baumanii*) was a rare etiology of neonatal sepsis. Makhoul et al., in 2002 and Jiang et al., in 2004 mentioned this organism as one cause for LOS. (Makhoul et al., 2002; Jiang et al., 2004) Two other papers reported *Acinetobacter* in outbreaks in nursery in USA. (Stone and Das, 1985; McDonald et al., 1998; Klein 2001) Moreover, *Acinetobacter* was known as an etiology for hospital infection in other age group and the trend increased.(Prescott et al., 2005) Among all outbreak in NICU, *Enterobacteriaceae* (*Klebsiella* spp., *Serratia* spp., *Enterobacter* spp., *Escherichia* spp., *Salmonella* spp., and *Citrobacter* spp.) were the predominant group. In an extensive study of 239 NICU outbreaks, *Acinetobacter* played role in only 13 (5.4%) of them. (Gastmeier et al., 2007)

Clinical manifestations of bacteremia due to *Acinetobacter* spp. are not unique. The frequency of occurrence of nosocomial infections by this organism is not easy to assess because the pathogenic role often has been underestimated.(Powell and Marcon 2003; Correa 2004)

One of VEOS patients had *Acinetobacter* infection revealed by hemoculture (4 others VEOS patients were : 2 *S. aureus* and 2 GBS which most probably were originated from the mother). Two other patients had *Acinetobacter* on the second day of age.

In the Phillipines, *S. pneumoniae*, *S. aureus*, and other *Streptococcus* species were known as important pathogens.(Quiambao et al., 2007) Only *S. aureus* had a high number in this study. In India, the most common organisms were *Staphylococcus* (*S. aureus* and CONS), *Streptococcus*, and *Enterococci*. Gram positive cocci constituted 48.3% of all isolates.(Upadhyay et al., 2006) EOS in VLBW in USA were mostly caused by gram negative bacteria (53%) with *E. coli* as the main organism.(Stoll et al., 2005)

As predicted, the incidence of GBS was rare, similar with other reports from developing countries.(Klein 2001; Vergnano et al., 2005)

For late sepsis, CONS and *S. aureus* were the most common. Since the mortality by CONS was low, the gram negative bacteria caused higher death rate.(Jiang et al., 2004)

A 10-year prospective surveillance in Brazil revealed 51.6% episodes of neonatal infection caused by gram negative rods (mainly *Klebsiella* spp. and *E. coli*).(Couto et al., 2007) In Ramatibodhi Hospital, 13 of 17 proven LOS patients were caused by gram positive bacteria, mostly the CONS.(Okascharoen et al., 2002) The same result was reported from Korea (62%), Bahrain (more than 50% by gram positive organisms), and Taiwan (CONS was the highest, 20.1%).(Jiang et al., 2004; Bindayna et al., 2006; Park et al., 2007) Report from USA found the percentage of gram positive in LOS among VLBW of 70%.(Stoll et al, 2002)

Among the death patients, only 1 was caused by gram positive bacteria (CONS, EOS group). The causes for others were *K. pneumoniae* (5), *Acinetobacter* (2), *Pseudomonas* (3), *Serratia*, *Citrobacter*, *H. influenzae* and *Enterobacter*.

Among the gram negative organisms, *E. coli* showed increasing trend. (Alarcon et al., 2004; Bindayna et al., 2006; Schrag et al., 2006; Soto et al, 2007) In this study the incidence of *E. coli* was 9.8%, similar with in Bahrain (10%).(Bindayna et al., 2006) The raising trend was probably caused by the effect of intrapartum ampicillin as prevention of GBS in developed countries.(Joseph et al., 1998; Alarcon et al., 2004; Schrag et al., 2006)

The percentage of ESBL producing *Klebsiella* among all *K. pneumoniae* in sepsis cases was 31.8%, lower than 58% in India.(Jain et al., 2007)

There was only one case of *S. pneumoniae* in this study and no *Salmonella* spp. found.

5.3. THE MODEL

5.3.1. Sample

All possible proven neonatal sepsis patients during 3 fiscal years period were included in this study. The initial calculation of sample size was 80 sepsis patients. However, since the sample should be divided into 2 categories, the sample size for each category would be smaller and might cause incorrect effect to the result of the study. The possibility for adding some more samples was constrained by limited time and other resources.

Nevertheless, this study had a larger sample size than previous studies. NOSEP Score by Mahieu et al. (2000) used 43 episodes of proven episodes and 104

suspected sepsis episodes, but did not use non suspected sepsis patients.(Mahieu et al., 2000) Okascharoen et al. (2005) used 1870 neonates, with only 17 proven sepsis patients (Okascharoen et al., 2005); Singh et al. (2003) used 30 episodes of definite, 17 most probable, and 58 non sepsis patients in the study.(Singh et al., 2003)

5.3.2. Dependent Variable

The dependent variable for this study was proven neonatal sepsis. The proof was mostly based on the culture results, particularly hemoculture. All sepsis patients without proof were excluded. Clearly defined outcome variable is an important requirement.(Guyatt 2006)

Culture is a gold standard for neonatal sepsis. However,the use of proven sepsis only may result in under-estimation of true incidence since it will be positive for only 35-41%.(Escobar et al., 2000; Klein 2001; Upadhyay et al., 2006) Negative culture result occurs sometimes even some patients are presented with highly suggestive clinical signs and symptoms for infections. The following factors affect the likelihood of recovering at least 1 colony-forming unit in the blood culture specimen : the number of bacteria in the blood at the time the culture specimen was obtained, the culture techniques, the volume of blood obtained, dilution (the ratio of blood to culture medium in the blood culture bottle), number of cultures taken, choice of blood culture bottles and system, and the usage of antibiotics.(Buttery 2002; Polin et al., 2005)

In an in vitro study, Schelonka et al. (1999) demonstrated that when the bacteria in the blood was 4 colony forming units/ml or less, a culture volume of 0.5 ml failed to detect at least 13% of the time. When the original colony count is 1 CFU/ml, the blood culture failed to detect bacteremia in almost 60% of the time. Sending 1 ml of blood for culture would improve the recovery rates to 98% and 63% if the original colony counts were 4 and 1 CFU/ml, respectively.(Schelonka et al., 1999; Polin et al., 2005) Unfortunately, the volume of neonatal blood sent for culture was frequently inadequate.(See 2005) Moreover, low level bacteremia was common in infants' patient population.(Kellog et al., 1997)

Another controversy is the time required for blood culture results to become positive. This is particularly relevant for the asymptomatic infants. In a study by

Kumar et al. in 2001, 97% of definite and possible bacterial pathogens were detected by 48 hours.(Kumar et al., 2001) Garcia-Prats et al. (2000) found that virtually all cultures grew clinically significant gram positive and negative organisms by 36 hours.(Garcia-Prats et al., 2000; Polin et al., 2005)

There are few clinical data on the effect of blood volume alone on blood culture outcome in newborns. In the United Kingdom, reported volumes per culture drawn vary from 0.3 ml to 0.66 ml, all well under the lower limit of 1 ml recommended by pediatric blood culture bottle manufacturers. Changes in the spectrum of microorganisms since 1970s (with high load of *E. coli*) to an increase in gram positive isolates (particularly CONS) has prompted investigators to revisit the small amount of blood needed for culture. (Buttery 2002)

The natural bactericidal activity of blood reduces the viability of organisms that can be recovered from blood cultures. This is due to innate immunity (including complement, phagocytic WBC, and lysozyme), acquired immunity to previously encountered pathogens, and residual antibiotics in the blood. The protective effect of liquid culture media results from dilution of bactericidal activity by the medium and binding of antibiotics by resins in many media. Different culture systems may also produce different sensitivities of detection.(Buttery 2002)

There are no neonatal data concerning the number of cultures taken. Usual practice is to take only 1 blood culture before starting antibiotic treatment. In children, raising the number of blood cultures to two or three bottles whether from 1 or more sites, does increase yield.(Buttery 2002)

Regarding the blood vessel choice, peripheral venous or arterial punctures are optimal, with no advantage of arterial cultures. False positive results may occur if sampling is from indwelling culture devices. Skin preparation before culture is also important.(Buttery 2002)

The neonatal sepsis patients without any proof were excluded from this study to avoid incorporation bias. This bias would appear if the possible predictive factors become part of diagnostic criteria. This incorporation of the test into the reference standard is likely to inflate the estimate of the test’s diagnostic power. It makes the reference standard to be dependent on the test. In usual practice, neonates with highly suspected clinical signs and symptoms plus suggestive laboratory results are often

diagnosed as having clinical sepsis even when the culture is negative.(Lijmer et al., 1999; Whiting et al., 2004)

This study did not include neonates with sepsis suspicion. Including only specific group of patients would overestimate the effects of predictive parameters. Developing a prediction score without including all neonates with many risk factors will result in poor performance of prediction score in subsequent validation studies.(Mahieu et al., 2000; Okascharoen et al., 2005)

5.3.3. Independent Variables

Independent variables in the study originated from some previous studies on predictive model for neonatal sepsis, and some scores for neonatal morbidity and mortality. The independent variables were added by some changes of continuous variables into qualitative forms. Others were made from unification of some variables. Abnormal renal function test, for example, represented abnormality in BUN and / or creatinine.

In total there were 144 variables in the beginning of this study. They were classified as risk factors/history, clinical conditions, laboratory data, and treatment modalities, as suggested in some previous reports.(Prober 2003) NOSEP Score by Mahieu et al. (2000) used 80, Okascharoen et al. (2005) had 34, WHO study in 1998 used at least 49, and Singh et al. (2003) used 16 variables (all were clinical signs).(Harell et al., 1998; Mahieu et al., 2000; Singh et al., 2003; Okascharoen et al., 2005)

Some newer laboratory examinations such as procalcitonin were not included because of availability reason.

5.3.4. Missing Data

Variables with more than 20% missing data, especially for the sepsis patients, were excluded in the first stage of analysis. Two reasons of high rates of missing data were the variables were not routinely done or they were not important.(Richardson et al., 1999) This step eliminated 33 and 25 variables in the early and late sepsis group, respectively.

There are various methods of handling missing data, namely: complete or available case analysis, the missing indicator method, and overall mean imputation.(Donders et al., 2006) In this study, for the remaining variables the missing data were corrected by 2 methods. For the non-sepsis patients, the mean of normal values were used. The patients were considered as “healthy enough” so some certain tests were not necessary, unless other reasons were stated in the medical record.(Richardson et al., 1999) Many mortality scores used the same considerations.(Shann et al., 1997; Richardson et al., 2001)

For the sepsis patients, the imputation method was used. The method is based on the idea that any subject in a study sample can be replaced by a new randomly chosen subject from the same source population. Imputation of missing data on a variable is replacing that missing by a value that is drawn from an estimate of the distribution of this variable. In single imputation only one estimate is used. In multiple imputation various estimates are used, reflecting the uncertainty in the estimation of this distribution. Under the general conditions of so-called missing at random and missing completely at random, both single and multiple imputations result in unbiased estimates of study associations.(Donders et al., 2006; Moons et al., 2006)

5.3.5. Univariate Tests

To reduce the number of predictor variables and to do the statistical selection, some univariate tests were used as appropriate. In those tests $p<0.1$ was used although some other models used $p<0.2$.(Richardson et al., 2001; Shapiro 2006, Guyatt 2006) NOSEP Score used $p<0.1$ and 41 variables were entered in the next step.(Mahieu et al., 2000) Okascharoen et al (2005) used $p<0.05$ and 20 of 34 variables were moved to the multivariate analysis.(Okascharoen et al., 2005) Singh et al. did not use univariate test for the study. The selection of variables was based on positive likelihood ratio.

The results of this process in this study were 53 (17 risk factors/history, 12 clinical condition, 22 laboratory, and 2 treatment modalities) and 68 (21 risk factors, 11 clinical condition, 34 laboratory, and 2 treatment modalities) variables in the early and late sepsis group, respectively.

5.3.6. Multivariate Analysis

Multivariate analysis used multiple logistic regression because the outcome variable was dichotomous and this test was easy.(Shapiro 2006; Guyatt 2006) Not all variables were inserted into multivariate analysis. Reselection process was done based on clinical judgment, colinearities (more than 1 variables measure the same thing), similarities, and performances.

Some less important variables such as education, occupation, and the environment were not used in the next step. If both continuous and qualitative data were present, the qualitative would be chosen because of practicability reason. The use of dichotomized data was also accurate and more useful in clinical practice. The original continuous data in NOSEP score derivation did not improve the accuracy of the global scoring system.(Mahieu et al., 2000)

In this study BUN was preferable than RFT since it needed only one examination. The same reason was applied for blood pressure. If more than one choice were available, all variables were tried one by one several times.

Gestational age did not pass the univariate test but this variable was tried to enter the multivariate analysis because of its clinical significance.(Okascharoen et al., 2005; Newton 2005) However, this variable still could not stay in the multiple logistic regression result. Some other significant risk factors could not enter the multivariate analysis probably because of the selection of control group. Most of the neonates in the control group had the same characteristic with the sepsis. The choice of non sepsis neonates would influence the univariate and multivariate results.

The final model for early and late sepsis group were selected based on the variable composition, clinical judgment, and performance of area under the ROC curve.(Higgins et al, 2000; Okascharoen et al., 2005)

5.3.7. The Equations and the Components

For early sepsis the equation used 5 variables (1 risk factors, 2 clinical conditions, and 2 laboratory data). Length of hospital stay was divided into 2 categories : 1-3 and 4-7 days. The longer the neonates stay in hospital the more risks

to develop nosocomial infection and sepsis. This variable had a highest adjusted OR in the model.

Poor feeding was selected, instead of lethargy, because of easiness in diagnosis. Sepsis, like many other problems in neonates, would cause problems in feeding. Since the earliest signs of sepsis are often subtle and non specific, poor feeding, diminished responsiveness, and “not looking well” may provide the only evidence that infection is present.(Klein 2001)

The oxygen saturation could be assessed by using oxymetri device without blood examination. Signs of respiratory distress are common and important findings in the infants suspected of having sepsis.(Klein 2001)

Two complete blood count parameters retained in the model : thrombocytopenia and leucopenia. Thrombocytopenia accompanying bacterial infection is thought to be caused by direct effect of bacteria or bacterial products on platelet and vascular endothelium leading to increased aggregation and adhesion or by increased platelet destruction caused by immune mechanisms.(Weinberg and Powell, 2001) In VLBW, fungal and gram-negative infections are more frequently associated with thrombocytopenia and it is more prolonged compared with gram positive pathogens.(Guida et al., 2003)

Thrombocytopenia (<150 000/cmm), leucopenia (<5000/cmm) and I:T ratio >12% are accurate cutoff values for early onset neonatal sepsis.(Phillip and Hewitt, 1980; Rodwell et al., 1988; Berger et al., 1995; Manucha et al., 2002) In this study I:T ratio could not be used since band count was not routinely done outside the working hours.

For late sepsis group the equation used 6 variables (4 clinical conditions and 2 laboratory data). Two of these variables were the same with early sepsis model (poor feeding and abnormal oxygen saturation).

Abnormal heart rate had the second highest adjusted OR after the abnormal temperature. Abnormal heart rate characteristics (reduced variability and transient decelerations) occurred early in the course of neonatal sepsis. These abnormalities were present 12-24 hours before the clinical diagnosis of sepsis. There was no difference between positive and negative blood cultures of these patients group. This method was studied extensively by Griffin et al. in 2001 and 2003 (external

validation).(Griffin et al., 2001; Griffin et al., 2003) In this study, the normal value was more simple and not using a sophisticated method. Tachycardia, arrhythmia, and poor peripheral perfusion that occur in the absence of congenital heart disease are sensitive signs of sepsis.(Klein 2001)

Abnormal temperature had the highest adjusted OR in the model. This was the most frequent clinical feature in some studies.(Fanaroff et al., 1998; Mahieu et al., 2000; Okascharoen et al., 2005) For term infant, hyperthermia had highly predictive parameter. Some studies showed more than 50% of the sepsis patients had fever, while hypothermia was found in only 15% infants.(Klein 2001; Saez-Llorens and McCracken Jr., 2004) No infant with hypothermia in this study developed late onset sepsis, and this was similar with the result from Okascharoen et al. (2005). There are no reported prospective studies of temperature in neonates which included preterm and term infants and that used data from blood culture to identify bacterial pathogens. Temperature abnormality in preterm infants frequently results from environmental influence rather than infection.(Klein 2001; Okascharoen et al., 2005) With the introduction of isolette care of the premature infant to maintain an optimal thermic environment, thermoregulatory disturbances commonly become obvious when the nurse reports the need to make frequent changes in the thermostat to accommodate the infant's loss of regulatory control.(Saez-Llorens and McCracken Jr., 2004) Murki et al. (2006) used the difference between rectal and sole temperature as a marker for neonatal sepsis which could possibly be used in the future study.(Murki et al., 2006)

Despite their high odds ratio, abnormal heart rate and abnormal temperature parameters should be used cautiously because of the very wide 95% confidence interval.

Abnormal leucocytes was determined by Manroe's criteria.(Manroe et al., 1979) Leucocytes (total WBC count) is one of the most common test for evaluation of bacterial infection. Criteria by Manroe was still used by some reference books despite some weaknesses such as depending on the infant's age, gestational age, and the blood vessels.(Manroe et al., 1979; Schelonka et al., 1994) Manroe et al. reported a study from 585 peripheral blood counts obtained from 304 normal neonates and 320 counts from 130 neonates with perinatal complications. The standard was not

originally intended as a septicemia screening tool but has been used widely later for this purpose.(Manroe et al., 1979; Prober 2003)

Abnormal pH – mostly acidosis - would accompany hypoxemia. Metabolic acidosis is most commonly seen as a consequence of lactic acid accumulation from anaerobic metabolism in hypoxic infants.(Bell and Oh, 2005)

The NOSEP score had 5 final variables (1 risk factor, 1 clinical condition, and 3 laboratory data). The model from Okascharoen et al. had 6 variables (1 risk factor, 3 clinical conditions, and 2 laboratory data) and Singh et al. used 7 final variables (all clinical conditions).(Mahieu et al., 2000; Singh et al., 2003; Okascharoen et al., 2005) The older models by Phillip and Hewitt (laboratory data only), St. Geme et al.(perinatal risk factors only), Rodwell et al.(hematology only), and Singh et al.(perinatal risk factors only) used 5, 5, 7, and 6 variables, respectively.(Phillp and Hewitt, 1980; St Geme et al., 1984; Rodwell et al., 1988; Singh et al., 1994) WHO scoring system had 13 variables and used a nomogram since the system was more complicated.(Harrell et al., 1998)

Variables from many scoring systems were listed in Appendix D. None of those models above used the probability equations. All of them used scores.

5.3.8. The Score

Changing the equation into the scoring system will make the usage of the model easier. This study provided 2 scoring systems, each for early and late neonatal sepsis. In comparison with the probability from the equation, the scoring system had good results as showed in the scatter diagrams.

Similar with the process in multivariate analysis, changing the equation into the scoring system should be tried several times. The regression coefficients were used to determine the score.(Moons et al., 2006) At least 4 possibilities of rounding the coefficients were tried for each group. Different score would produce different performance of the result. The best system was chosen based on the AUC of ROC curve and other performance indicators. The final scoring system for EOS and LOS had AUC of 93.6% and 96.6%.

Maximum score for EOS and LOS were 14 and 23, respectively. Maximum score from other systems were : 9 (Mahieu), 15 (Okascharoen), 7 (Singh et al.,

2003), 7 (Rodwell), and 13 (WHO). (Rodwell et al., 1988; Harrell et al., 1998; Mahieu et al., 2000; Singh et al., 2003; Okascharoen et al., 2005)

5.3.9. Performance of the Equation and Score

Receiver Operating Characteristic (ROC) is a useful tool for evaluating the performance of diagnostic tests and more generally for evaluating the accuracy of a statistical model such as logistic regression that classifies subjects into 1 of 2 categories. Its function as a simple graphical tool for displaying the accuracy of a medical diagnostic test is one of the most well known applications of ROC curve analysis. A ROC curve is a plot of sensitivity on the y axis against (1-specificity) on the x axis for varying values of the threshold t. The area under the ROC curve is a summary measure that essentially averages diagnostic accuracy across the spectrum of test values.(Zou et al., 2007)

For EOS, the AUC of ROC of the equation was 87.7% which mean this equation had 87.7% chance of correctly classify sepsis and non sepsis. The AUC of the score was higher. For LOS, the AUC were 95.6% for the equation and 95.5% for the score.

The model by Okascharoen et al. had AUC of ROC of 99% meanwhile NOSEP score had 82%. The modification of NOSEP (NOSEP-NEW-I and NOSEP-NEW-II) had AUC of 71% and 82%.(Mahieu et al., 2000; Mahieu et al., 2002; Okascharoen et al., 2005)

The sensitivity and specificity of EOS equation were above 70% for the probability cutoff 20%, meanwhile the cutoff for the score was 1. For LOS the cut off were 20-40% (equation) and 2-3 (score). However, the choice of cutoff (including the PPV, NPV, LR+, and LR(-)) will depend on the purpose of usage. For the balanced sensitivity and specificity, the choice should have above 70% value. If the purpose is not to miss any proven bacterial neonatal sepsis then the choice is 100% of sensitivity and NPV. This choice principle is also applied for other cases such as bacterial meningitis.(Nigrovic et al., 2002; Singh et al., 2008)

5.3.10. Validation

Validation is a requirement since such models do not always work well in practice. The idea of validating a prognostic model is generally taken to mean establishing that it works satisfactorily for patients other than those from whose data were derived.(Altman and Royston 2000; McGinn et al., 2000; Reilly and Evans, 2006)

Since some limitations in the time and other resources, validation was not done prospectively and completely in this study. About 35% patients from the original sample sets were used randomly, and this process was repeated twice for each group. Once again, AUC of ROC curve was used for discrimination. Hosmer-Lemeshow test was used for calibration.(Lemeshow and Hosmer, 1982)

All four AUC of ROC curve for score were above 80%. All four p results of Hosmer-Lemeshow test for score were above 0.05. That means no statistically significant difference between validation and original group (the scoring system was good).

Some other predictive models were validated externally by the original model developer team. The external validation of NOSEP produced AUC of 66% and the author then made a modification to the original score. However, another validation from Turkey concluded that the result was not good enough and “currently there seems to be no reliable tool for early diagnosis of sepsis in neonates….”.(Mahieu et al., 2002; Dalgic et al., 2005)

External validation of Okascharoen’s model was done in Canada. The result of AUC was 78%. The model of Singh et al was validated specifically for 1000-2500 grams neonates. The author presented the performance by sensitivity, specificity, PPV, NPV, LR+, and LR(-) without the AUC. The conclusion of this study was “the clinical score in combination with sepsis screen result can be used by clinicians to rule out sepsis”.(Mahieu et al., 2002; Okascharoen et al., 2007; Kudawla et al., 2008)

5.3.11. Difficulty in Comparison

Although some steps in the development were comparable, proper comparison with some other models could not be done easily since each model differ

from each other in term of age criteria, type of variables, validation process, and the purpose of the score.

NOSEP score and Okascharoen's score use criteria of 3 days of age to determine early or late. The WHO system and Bachur and Harper used age of 0-90 days. Rodwell et al. only used hematology parameter while Singh et al. (2003) used only clinical conditions. In the Singh setting, the prevalence of infection was very high, 44.5% with majority of *S. aureus* (30%). The score by Phillip and by Singh (2003) use the term "screening" for their score.(Phillip and Hewitt, 1980; Rodwell et al., 1988; Harrell et al., 1998; Mahieu et al., 2000; Bachur and Harper, 2001; Singh et al., 2003; Okascharoen et al., 2005)

5.4. RISK FACTORS

Among demographic and history (antepartum / intrapartum / postpartum) risk factors for sepsis, 9 factors were significantly different between sepsis and non sepsis neonates for EOS group and 11 for LOS. Variable with the highest odds ratio was length of hospital stay before sepsis, especially for EOS. Other factors with high odds ratio for EOS were high risk pregnancy and icterus after birth. For LOS, factors with high significant odds ratio were high risk pregnancy, intracranial hemorrhage, and resuscitation. Except for length of hospital stay for EOS, none of these factors appeared in the model.

5.5. CLINICAL CONDITION

Among the clinical conditions, lethargy, poor feeding, abnormality of heart rate, abnormality of temperature, respiratory insufficiency, and hypoxemia were statistically significant different between sepsis and non sepsis patients in both EOS and LOS groups. Factors with the highest odds ratio for sepsis in EOS group were apnea episode, hypoxemia, lethargy, and poor feeding. For LOS, variables with the highest odds ratio were abnormal heart rate, abnormal temperature, and poor feeding. Only lethargy and apnea episode could not enter the final models.

5.6. LABORATORY DATA

Most of the laboratory parameters were statistically significant different between sepsis and non sepsis patients in both EOS and LOS groups. Parameters with the highest odds ratio for EOS were leucopenia, abnormal BUN, and thrombocytopenia. Meanwhile, the parameters for LOS were abnormal CSF glucose, abnormal pH, and abnormal leucocytes (Manroe's criteria). Abnormal BUN and CSF glucose could not enter the final models.

5.7. TREATMENT PATTERN

Immunoglobulin, blood component, vascular catheter, and exchange transfusion were 4 treatment modalities that significantly different between sepsis and non sepsis patients in the EOS group. For LOS group, the differences were found regarding immunoglobulin, blood component transfusion, vascular catheterization, and total parenteral nutrition. The highest odds ratio were found for the usage of central catheter for both EOS and LOS, and total parenteral nutrition for LOS. There were no items from treatment modalities entered the final models.

Among all sepsis patients, 33 (73.3%) in EOS and 41 (78.9%) in LOS group received ampicillin. Combination of ampicillin and gentamicin is still recommended for early onset sepsis, despite the guidelines has already been 3 decades old. Cephalosporins are not active against *Listeria* or *enterococci* and should not be used without concomitant administration of ampicillin.(Klein 2001; Edwards and Baker 2003; WHO 2003; Saez-Llorens and McCracken Jr., 2004) One study in 2006 found that for patients receiving ampicillin, the concurrent use of cefotaxime during the first 3 days after birth either is a surrogate for an unrecognized factor or as itself associated with an increased risk of death, compared with the concurrent use of gentamicin.(Clark et al., 2006)

Treatment for *Acinetobacter* spp, particularly *A. baumanii*, has become more complicated by the rapid increase in resistance to the antibiotics commonly used in hospitals. The carbapenems, imipenem and meropenem, appear to be the most active agents against *A. baumanii* but recent reports have found more than 10 percent of such strains to be resistant to these antibiotics in some areas.(Powell and Macron 2003; Correa 2004)

Because LOS is more heterogenous in its epidemiology than is early onset disease and may reflect maternal, family, community, or nosocomial sources for the infecting pathogen, the organisms involved cover a broad taxonomic spectrum. As a result, empirical antimicrobial regimens vary. Selection of empirical antibiotics regimens can be more difficult for a septic premature infant who has had a prolonged hospitalization, previous antibiotic administration, possible prolonged tracheal intubation, and placement of a central or peripheral intravascular catheter.(Saez-Llorens and McCracken Jr., 2004),

A small number of studies have examined the use of IVIG as an adjunct to antibiotic treatment in infants with suspected or proven sepsis. Unfortunately, the quality of these studies were poor.(Shaw et al., 2007) The Cochrane meta-analysis showed a 10% reduction in mortality. However, the 95% CI for the number needed to treat was wide (NNT 10; 95% CI 5-200), and there was no statistically significant reduction in mortality after treatment with IVIG in cases where infection was proven.(Ohlson and Lacy 2004; Shaw et al., 2007) One study from Turkey (2006) compared IVIG and exchange transfusion. There were no differences between the initial and 24 hour IgG levels in the IVIG, exchange transfusion, and control groups. IgM levels rose significantly 12 hours after exchange transfusion and elevated IgM persisted for over 24 hours.(Gunes et al., 2006)

Labenne et al. (2007) assessed the compliance with- and the effect of noncompliance with guidelines intended to limit the use of broad-spectrum antibiotics and to reduce the duration of the antibiotic therapy. As conclusion, reducing antibiotic therapy duration did not increase the risk of infectious relapse and might decrease the incidence of late onset infection.(Lebenne et al., 2007)

5.8. SEASONAL VARIATION

There was no significant statistical difference between the month of early and late onset neonatal sepsis incidence in QSNICH. However, there was statistical significant difference between the number of sepsis patient during the first semester of the year (January-June) and the second semester. The higher total number of neonatal patients during this period might have influence.

In some countries, there were seasonal influences by the incidence of preterm birth (or total birth). Since most of neonatal sepsis patients were preterm, the change of incidence probably will affect the incidence of neonatal sepsis. However, the report of the relation of season with incidence of neonatal sepsis was lacking.(Bang et al., 2005b)

In India and Bangladesh, lowest birth incidence was in October-December (post monsoon period).(Hort 1987; Gohil 2006) In London, the highest incidence of preterm was during winter.(Lee et al., 2006) In Japan and USA there were 2 peak times of preterm birth, those were in summer and winter.(Cooperstock and Wolfe, 1986; Matsuda and Kahyo, 1992) In Gambia, the peaks of preterm birth incidence were also 2 times, July (beginning of hungry season) and October (end of hungry season).(Rayco-Solon et al., 2005) In Harare Zimbabwe, the peak of preterm birth was on the late rainy season.(Friis et al., 2004) One other study from USA stated that there was no seasonal difference for preterm birth incidence.(Konte et al., 1988)

5.9. LIMITATIONS OF THE STUDY

The main limitation of this study was its retrospective design. Information bias cannot be avoided by using that design and by taking data from medical records. The sample size of the study was limited, since total sample had to be divided into 2 groups.

The missing data (as an unavoidable part of retrospective design study) was also the limitation since any methods, however perfect, can lead to biased estimates of the odd ratio and the model performance in predictive models.(Gorelick 2006) The choice of patients in the control group (non sepsis) might also affect the result of the study. For example, in this study, most of the non sepsis cases had hyperbilirubinemia. The result for icterus variable might be different if predominant diagnoses were other diseases.

The chosen outcome was only proven sepsis. This could result in under estimation of true incidence. However, including unproven sepsis would cause incorporation bias.

Lastly, validation of a new sample set was needed, either in the same setting or others. It is recommended to perform the validation process prospectively.

5.10. FUTURE DIRECTION

A validation with a new sample set is needed to test the usage of the model in clinical practice. In this validation, larger sample size will be needed.

A modification by using some other diagnostic methods (such as CRP and I:T ratio) should be anticipated in the near future.

CHAPTER VI
CONCLUSION

The conclusions of the study were :

1. Two predictive models were produced, each for early and late onset proven neonatal sepsis. In each model, there were equation and scoring system. Equation and scoring system for early and late onset proven neonatal sepsis were shown in the boxes.
2. Performances of all equations and scoring systems during the development and validation phases were good.
3. Risk factors which had the highest odds ratio for early onset proven neonatal sepsis were : length of hospital stay before sepsis, high risk pregnancy and icterus after birth. Risk factors which had the highest odds ratio for late onset proven neonatal sepsis were : length of hospital stay before sepsis, high risk pregnancy, intracranial hemorrhage, and neonatal resuscitation.
4. Among all clinical conditions in this study, the highest odds ratio were found on apnea episode, hypoxemia, lethargy, and poor feeding (all for early onset proven neonatal sepsis) and abnormal heart rate, abnormal temperature, and poor feeding (all for late onset proven neonatal sepsis).
5. For laboratory parameters, the study revealed that the highest odds ratio for early onset proven neonatal sepsis were leucopenia, abnormal BUN, and thrombocytopenia. For late onset proven neonatal sepsis, they were abnormal CSF glucose, abnormal pH, and abnormal leucocytes (Manroe's criteria).
6. All neonatal sepsis patients received antibiotics. Ampicillin is the most common used antibiotic.
7. There was no statistically significant difference between the incidence of early and late onset proven neonatal sepsis by month. There was a statistically significant difference between the incidence of sepsis in the first and second semester.

FOR EARLY ONSET PROVEN NEONATAL SEPSIS

THE EQUATION :

Y = (2.364 * length of hospital stay before sepsis)
+ (1.268 * poorfeeding)
+ (1.896 * abnormal oxygen saturation)
+ (1.540 * thrombocytopenia) + (1.872 * leucopenia)
- 2.895

THE SCORE :

Length of stay before sepsis
- < 4 days = 0
- 4-7 days = 2

Poorfeding
- Yes = 1
- No = 0

Abnormal oxygen saturation (< 92%)
- Yes = 2
- No = 0

Thrombocytopenia (<150 000 /cmm)
- Yes= 1
- No = 0

Leucopenia (≤ 5000 / cmm)
- Yes = 2
- No = 0

FOR LATE ONSET PROVEN NEONATAL SEPSIS

THE EQUATION :

Y = (2.398 * poorfeeding) + (3.087 * abnormal heart rate)
+ (3.995 * abnormal temperature)
+ (1.387 * abnormal oxygen saturation)
+ (1.786 * abnormal leucocytes)
+ (2.479 * abnormal pH) – 4.328

THE SCORE :

Poorfeeding
- Yes = 2
- No = 0

Abnormal heart rate (100 – 180 x/minute)
- Yes = 3
- No = 0

Abnormal temperature (36 – 37.9^{o}C)
- Yes = 4
- No = 0

Abnormal oxygen saturation (< 92%)
- Yes = 1
- No = 0

Abnormal leucocytes
< 7 days of age : 9000 – 30 000 /cmm
7-14 days of age : 5000 – 21 000 /cmm
> 14 days of age : 5000 – 20 000 /cmm
- Yes = 2
- No = 0

Abnormal pH (7.27 – 7.45)
- Yes = 2
- No = 0

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APPENDIX

APPENDIX A

CASE RECORD FORM

Sample Number : _ _ _
Date of collecting : _ _ / _ _ / _ _

By :

A	Basic – Demographic Data					
1	Date of admission (dd/mm/yy)	:	_ _ / _ _ / _ _			
2	Date of discharge (dd/mm/yy)	:	_ _ / _ _ / _ _			
3	Date of birth (dd/mm/yy)	:	_ _ / _ _ / _ _			
4	Date of dx of sepsis(dd/mm/yy)	:	_ _ / _ _ / _ _			
5	Age on admission (days)	:	[__ __]			
6	Sex	:	□ M □ F			
7	Referred from	:	□ Rajvithi Hospital	□ Home		
			□ Clinic	□ Other hospital	□ Others	
8	Outcome	:	□ Death	□ Alive		
			□ Discharge on request			
9	Admission Diagnosis	:	□			
			□			
			□			
10	Final (Co-morbid) Diagnosis	:	□			
			□			
			□			
			□			
			□			
B	Maternal History – Antenatal					
1	Frequency antenatal care	:	□1 □ 2 □3 □ 4	□ >4 None		□NA
2	Location of antenatal care	:	□ Hospital	□ Clinic		□NA
				□ Others		
3	Mother's + Father's race	:	□ Thai □ Non Thai	□ Mixed		□NA
4	Mother's age (years)	:	[__ __]			
5	Smoking / Alcohol	:	□ Y □ N /	□ Y □ N		□NA
6	Genital disease of the mother	:	□ Smelly discharge	□ Tumor like		□NA
			□ Genital lesion	□ Others		
			□ None			
7	Other chronic disease of the mother	:	□ HIV/AIDS □ Syphilis	□ Diabetes	□ None	□NA
			□ Hepatitis B □ Others (define):			
8	High risk pregnancy	:	□ Y □ N			□NA
9	Long term drug used	:	□ Y □ N			□NA
10	Education of the mother	:	□ Elementary	□ High school		□NA
			□ University	□ None		
11	Occupation of the mother	:	□ Office □ Shop	□ Labor	□ None	□NA
			□ Government service	□ Others		
12	Environment	:	□ Slum □ Factory	□ Market	□ Others	□NA
13	Number of gravida	:	[__ __]			
14	Space from previous birth (yrs)	:	[__ __]			□NA

C	History – Natal			
1	Mode of delivery	:	□ Spontaneous □ Caesarian Section □ Forceps □ Vacuum □ Breech extraction Indications : ……	
2	Premature rupture of membrane >18 hrs	:	□ Y □ N	□NA
3	Chorioamnionitis	:	□ Y □ N	□NA
4	Smelly amniotic fluid	:	□ Y □ N	□NA
5	Color of amniotic fluid	:	□ Colorless □ Turbid □ Green □ Others	□NA
6	Fever of the mother	:	□ Y □ N	□NA
7	Complications of pregnancy	:	□ Antepartum bleeding □ Infection □ Eklampsia/Pre □ Placenta Previa □ None □ Other:	□NA
8	Location of birth	:	□ Hospital □ Home □ Others	□NA
9	Birth helper	:	□ Doctor □ Midwife □ Nurse □ Traditional helper □ Other	□NA
10	Steroid injection for the mother	:	□ Y □ N	□NA
11	Antibiotic for the mother	:	□ Y □ N	□NA
12	Singleton/gemelli	:	□ Singleton □ Gemelli	
13	Birth weight (g)	:	[ ]	
14	Gestational age (weeks)-Ballard	:	[ ]	□NA
15	Apgar score 1 minute	:	[ ]	□NA
16	Apgar score 5 minute	:	[ ]	□NA
17	Lubchenko	:	□ AGA □ SGA □ LGA	□NA
18	Intracranial hemorrhage	:	□ Y □ N	□NA
19	Umbilical cord	:	□ Fresh □ Not fresh	□NA
20	Resuscitation	:	□ Y □ N	□NA
21	If Yes, how many minutes ?	:	[ ]	
22	If Yes	:	□ O_2 mask □ Intubation	□NA
D	History - Postnatal			
1	Taking care by whom	:	□ Family □ Medical personnel □ Traditional helper	□NA
2	Location	:	□ Home □ Hospital/Clinics □ Others	□NA
3	Umbilical cord treatment	:	□ Alcohol / T Dye / Iodine / Chlorhexidine □ Others □ None	□NA
4	Icterus	:	□ Y □ N	□NA
5	Seizure	:	□ Y □ N	□NA
6	Fever	:	□ Y □ N	□NA
7	Breastfed	:	□ Y □ N	□NA
E	Clinical Manifestations			
1	Letargy / less movement	:	□ Y □ N	□NA
2	Poor feeding	:	□ Y □ N	
3	Cyanosis	:	□ Y □ N	□NA
4	Icterus	:	□ Y □ N	□NA
5	Sistolic (mmHg)- lowest	:	[ ]	□NA
6	Diastolic (mmHg) - lowest	:	[ ]	□NA
7	Heart Rate/Pulse (x/minute)	:	[ ]-highest [ ]-lowest	□NA
8	Temperature (^oC) ; rectal	:	[ ]-highest [ ]-lowest	□NA
9	Respiration rate (x/minute)	:	[ ]-highest [ ]-lowest	□NA

10	Respiration pattern	:	□ Shallow	□ Deep	□NA
11	Apnea episode	:	□ Y □ N		□NA
12	Grunting	:	□ Y □ N		□NA
13	Chest retraction	:	□ Y □ N		□NA
14	Seizure	:	□ Y □ N		
15	Capillary refill (seconds)	:	[ ]		□NA
16	Urine production for 24 hours (cc)	:	[ ]		□NA
F	Laboratory	:			
1	Hemoglobin (g/dl)	:	[ ]		□NA
2	Leukocyte (/cmm)	:	[ ]-highest	[ ]-lowest	□NA
3	Thrombocyte (/cmm)	:	[ ]		□NA
4	Immature neutrophil count	:	[ ]		□NA
5	Total neutrophil count	:	[ ]		□NA
6	ESR (mm/hour)	:	[ ]		□NA
7	pH	:	[ ]		□NA
8	PaO_2 (mmHg) - arterial	:	[ ]		□NA
9	pCO_2 (mmHg) - arterial	:	[ ]		□NA
10	HCO_3 (meq/liter)	:	[ ]		□NA
11	Base Excess	:	[ ]		□NA
12	O_2 saturation (%)	:	[ ]		□NA
13	FiO_2 (%)	:	[ ]		□NA
14	K (mmol/liter)	:	[ ]-highest	[ ]-lowest	□NA
15	Na (mmol/liter)	:	[ ]-highest	[ ]-lowest	□NA
16	Ca (mg/dl)	:	[ ]-highest	[ ]-lowest	□NA
17	Blood sugar (mg/dl)	:	[ ]-highest	[ ]-lowest	□NA
18	BUN (mg/dl)	:	[ ]		□NA
19	Creatinine serum (mg/dl)	:	[ ]		□NA
20	AST (SGOT) (U/l)	:	[ ]		□NA
21	ALT (SGPT) (U/l)	:	[ ]		□NA
22	Direct Bilirubin (mg/dL)	:	[ ]		□NA
23	Total Bilirubin (mg/dL)	:	[ ]		□NA
24	CRP I (mg/dL)	:	[ ]		□NA
25	CRP II (mg/dL)	:	[ ]		□NA
26	CSF-turbidity	:	□ Y □ N		□NA
27	CSF-open/closing pressure (cmH_2O)	:	[ ] [ ]		□NA
28	CSF-cell count (cell/ul)	:	[ ]		□NA
29	CSF-PMN : MN(%)	:	[ ]:[ ]		□NA
30	CSF-glucose (mg/dl)	:	[ ]		□NA
31	CSF-protein (mg/dl)	:	[ ]		□NA
32	CSF-gram stain (describe)	:	□ Neg □ Pos :		□NA
33	Urine-protein (mg/dL)	:	[ ]		□NA
34	Urine-glucose (+/-)	:	[ ]		□NA
35	Urine-sediment-leukocyte (/pf)	:	[ ]		□NA
36	Urine-sediment-erythrocyte (/pf)	:	[ ]		□NA
37	Urine-cast (describe)	:	[ ]		□NA

38	Latex (+/-) ; from (describe)	:	[ ] ; ……			□NA
	If +, microorganism	:	……			
39	Staining (+/-) ; from (describe)	:	[ ] ; ……			□NA
	If +, microorganism	:	……			
40	PCR (+/-) ; from (describe)	:	[ ] ; ……			□NA
	If +, microorganism	:	……			
40	Others antigen (+/-); microorganism	:	[ ] ; ……			□NA
41	Others antibody (+/-); microorganism	:	[ ] ; ……			□NA
42	Culture I - Source	:	□ Blood □Urine □ Tracheal □ Pus	□ Feces □ Others	□ CSF	□NA
43	Result (describe)		□ Neg □ Pos :			
44	Culture II - Source	:	□ Blood □Urine □ Tracheal □ Pus	□ Feces □ Others	□ CSF	□NA
45	Result (describe)		□ Neg □ Pos :			
46	Culture III – Source	:	□ Blood □Urine □ Tracheal □ Pus	□ Feces □ Others	□ CSF	□NA
47	Result (describe)		□ Neg □ Pos :			

G	Treatments					
1	Antibiotic I (name)	:	[ ]			
2	Duration (days) / Dosage (MKD)	:	[ ] / [ ]			
3	Antibiotic II (name)	:	[ ]			
4	Duration (days) / Dosage (MKD)	:	[ ] / [ ]			
5	Antibiotic III (name)	:	[ ]			
6	Duration (days) / Dosage (MKD)	:	[ ] / [ ]			
7	Immunoglobulin	:	□ Y □ N			
8	Blood Component	:	□ Y □ N			
9	Exchange Transfusion	:	□ Y □ N			
10	Artificial ventilation	:	□ Y □ N	□ CPAP		□NA
11	If yes, illness ?	:	□ RDS □ TTNP	□ MAS	□ Other	
12	Vascular catheter	:	□ Umbilical □ Venous	□ Arterial	□ None	□NA

Note :

Y	= Yes
N	= No
M	= Male
F	= Female
Neg	= Negative
Pos	= Positive
NA	= Data Not Available

APPENDIX B
DEFINITIONS OF SEPSIS

DEFINITIONS OF SEPSIS FROM LITERATURES

Several definitions of sepsis from literatures are described below.

Bacteremia : the presence of bacterial pathogen in blood.(Haque 2007)

Infection : a pathological process initiated by invasion of normally sterile site, fluid, or body cavity by pathogenic or potentially pathogenic organisms.(Haque 2007) A suspected or proven (by positive culture, tissue stain, or PCR test) infection caused by any pathogen or a clinical syndrome associated with high probability of infection. Evidence of infection includes positive findings on clinical exams, imaging, or laboratory tests.(Goldstein et al., 2005)

Systemic Inflammatory Response Syndrome (SIRS) : The presence of at least 2 of the following 4 criteria, one of which must be abnormal temperature or leukocyte count : (Goldstein et al., 2005; See 2005)

- Core temperature > 38.5 or < 36^{o}C
- Tachycardia > 2 SD or bradycardia < 10 percentile
- Mean respiratory rate > 2 SD
- Leukocyte count elevated or depressed for age or > 10% immature neutrophils

Fetal Inflammatory Response Syndrome (FIRS) : (Haque 2007)

- Tachypnea (respiratory greater than 60 breaths/minute plus either grunting / chest retractions or desaturations)
- Temperature instability (< 36^{o}C or >37.9^{o}C)
- Capillary refill time >3 s
- Total WBC (<4 x 10^{9} /L or >34 x 10^{9} /L)
- CRP >10 mg/dl or IL-6 or IL-8 >70 pg/ml
- Positive 16s RNA gene PCR

Table 46. Organ Dysfunction Criteria

Cardiovascular dysfunction	Despite administration of isotonic intravenous fluid bolus >40 mL/kg in 1 hr - Decrease in BP (hypotension) <5th percentile for age or systolic BP < 2 SD below normal for age OR - Need for vasoactive drug to maintain BP in normal range (dopamine >5 ug/kg/min or dobutamine, epinephrine, or norepinephrine at any dose) 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/hr Prolonged capillary refill : >5 secs Core to peripheral temperature gap >3^{o}C
Respiratory	- PaO_2/FiO_2 <300 in absence of cyanotic heart disease or preexisting lung disease OR - $PaCO_2$ >65 torr or 20 mmHg over baseline $PaCO_2$ OR - Proven need or >50% FiO_2 to maintain saturation $\geq$ 92% OR - Need for nonelective invasive or noninvasive mechanical ventilation
Neurology	- Glasgow Coma Scale $\leq$ 11 OR - Acute change in mental status with a decrease in GCS >3 points from abnormal baseline
Hematology	- Platelet count <80 000 /mm^3 or a decline of 50% in platelet count from highest value recorded over the past 3 days (for chronic hematology/oncology patients) OR - International normalized ratio >2
Nephrology	- Serum creatinine $\geq$2 times upper limit of normal for age or 2 –fold increase in baseline creatinine
Hepatology	- Total bilirubin $\geq$4 mg/dL (not applicable for newborn) OR - ALT 2 times upper limit of normal for age

Source : Goldstein et al., 2005

Table 47. Age-Specific Vital Signs and Laboratory Variables (Lower Values For Heart Rate, Leukocyte Count, and Systolic Blood Pressure are for The 5th and Upper Values for Heart Rate, Respiration Rate, or Leukocyte Count For 95th Percentile)

AGE GROUP	HEART RATE (BEATS/MIN)		RESPIRATORY RATE (BREATHS/ MIN)	LEUKOCYTE COUNT (LEUKOCYTES X 10^3/mm)	SYSTOLIC BLOOD PRESSURE (mmHg)
	TACHY CARDIA	BRADY CARDIA			
0 days – 1 week	>180	<100	>50	>34	<59
1 wk – 1 mo	>180	<100	>40	>19.5	<79
1 mo – 1 yr	>180	<90	>34	>17.5 or <5	<75
2-5 yrs	>140	NA	>22	>15.5 or <5	<74
6-12 yrs	>130	NA	>18	>13.5 or <5	<83
13 to <18 yrs	>110	NA	>14	>11 or <5	<90

Source : Goldstein et al., 2005, Gebara 2005

Sepsis : SIRS in the presence of or as a result of suspected or proven infection.(Goldstein et al., 2005) Sepsis also defined as the growth of bacteria in blood cultures in conjunction with clinical signs of systemic infection.(Edwards & Baker, 2003; Ronnestad et al. 2005)

Severe Sepsis : Sepsis plus 1 of the following : cardiovascular organ dysfunction or acute respiratory distress syndrome or 2 or more other organ dysfunctions (see Table 46 and 47).(Goldstein et al., 2005)

Septic Shock : Sepsis and cardiovascular organ dysfunction (see Table 46 and 47).(Goldstein et al., 2005) Severe sepsis with hypotension requiring fluid resuscitation and inotropic support.(Haque 2007)

Definite or Proven BSI / Sepsis : A positive blood culture or a positive PCR in the presence of clinical signs and symptoms of infections (see Table 48).(modified from Haque 2005)

Probable BSI / Sepsis : Presence of clinical signs and symptoms of infection (Table 48) and serological response to antigen / antibody plus abnormal non specific laboratory results when blood culture or PCR are negative.(modified from Haque 2007 and See 2005)

Possible BSI / Sepsis: Presence of clinical signs and symptoms of infection (Table 48) plus non specific laboratory markers of infections in the absence of positive blood culture or PCR.(modified from Haque 2007 and See 2005) The difference between probable and possible BSI is that indirect evidence of a pathogen (positive serology) is present in probable BSI / sepsis, which is absent in possible sepsis.(See 2005) There are also few others definitions especially before 2005.(Martius et al., 1999; Escobar et al., 2000)

Table 48. Clinical Signs and Symptoms and Laboratory Parameters for Diagnostic Criteria in Neonatal Sepsis

Clinical Variables	• Temperature instability • Heart rate>2 SD above normal for age (>180 beats/min, ≤100 beats/min) • Respiratory rate (>60 breaths/min) plus grunting/recession or desaturations • Lethargy / altered mental status • Glucose intolerance (plasma glucose >10 mmol/L) • Feed intolerance
Hemodynamic Variables	• BP 2 SD below normal for age • Systolic pressure <50 mmHg (newborn day 1) • Systolic pressure <65 mmHg (infants ≤1 month)
Tissue Perfusion Variables	• Capillary refill >3 secs • Plasma lactate >3 mmol/L
Inflammatory variables	• Leukocytosis (WBC count >34 000 x 10^9 /L) • Leukopenia (WBC count <5 000 x 10^9 /L) • Immature neutrophils >10% • Immature : total neutrophil ratio >0.2 • Thrombocytopenia < 100 000 x 10^9 /L • CRP >10 mg/dL or >2 SD above normal value • Procalcitonin >8.1 mg/dL or 2 SD above normal value • IL-6 or IL-8 >70 pg/mL • 16S PCR : positive

Source : Haque 2005

Early Neonatal Sepsis = Early Onset Sepsis : if diagnosed ≤ 7 days of life. This limitation will be suitable with definition of early neonatal death by WHO that is

death occurring during the first seven days of life.(WHO 2006) Some experts divide early onset into : very early onset sepsis (episodes diagnosed on the day of delivery), and early onset sepsis (episodes diagnosed from day 2 to 7 of life).(Ronnestad et al.,2005) Some others limit early onset septicemia to $\leq$ 48 hours of life (Mtitimila and Cooke, 2004; Stoll et al., 2005), and 5 days of life. (Bizzaro et al., 2005)

Late Neonatal Sepsis = Late Onset Sepsis : if diagnosed > 7 days of life (but the initial diagnosis should not more than 28 days).(Lukacs et al., 2004) The term "late onset sepsis" variably defined in the literature from >48 hours to beyond 7 days after delivery.(Okascharoen et al., 2005; Gordon & Isaacs, 2006; Datta et al., 2006) . The term "late late onset" septicemia is used for the age >30 days or even 3 months.(Bizzaro et al., 2005; Datta et al., 2006)

Clinical Sepsis : Sepsis in which blood cultures are not performed, not detected, or for which the physician institutes treatment for sepsis.(See 2005)

Primary BSI : BSI in babies without an identifiable source of infection.(Haque 2007)

Secondary BSI : BSI in babies caused by pathogens related to infection at another site.(Haque 2007)

No BSI : Absence of clinical signs and symptoms of infections and abnormal laboratory results.(Haque, 2005)

Nosocomial Infection : An infection that occurs more than 48 hours after admission in a baby who on admission did not have evidence of infection.(Haque 2007)

APPENDIX C

LIST OF RISK FACTORS FROM PREVIOUS PUBLICATIONS

Darmstad et al., 2000-all neonates

Maternal History

- Delivery conditions: hygiene, vaginal examinations, insertion of material into or cutting the vagina
- Fever
- Premature rupture of the membrane (<37 wk gestational age)
- Prolonged rupture of membranes (>12 h)
- Prolonged labor
- Evidence of chorioamnionitis (abdominally/pelvic/uterine tenderness, foul smelling amniotic fluid/vaginal discharge)
- Antibiotic received

Lott 2006

Maternal Factors

- Race
- Ethnic origin
- Socioeconomic status
- Substance abuse
- Maternal illness
- Maternal infection
- Compromised genital tract of the mother, ie leaking/ruptured membrane, colonization by bacteria
- Inadequate prenatal care
- Poor nutrition

Neonatal Factors

- Male sex
- Multiple gestation
- Galactosemia
- Congenital disorders of immune system
- Disruptions of skin or mucous membranes

Other Factors

- Environment
- Geographic region

Ronnestad et al, 2005 **VLBW** • Gestational age • Birth weight • Apgar score • Intrapartum antibiotics • Maternal antibiotics ≥72 hours of delivery • Rupture of membranes ≥24 hours • Clinical chorioamnionitis • Preeclampsia • Mode of delivery • Intrauterine growth • Surfactant • Early vasopressor treatment • Mechanical ventilation • CPAP • Endotracheal intubation • Initial treatment • Receiving antibiotics the 2nd day • Umbilical catheter • Central venous catheter • Transfusion • Intraventricular hemorrhage	**Escobar et al., 2000** **≥ 2000 grams** • Race • Maternal age • Maternal chorioamnionitis • Length of ROM • Antepartum temperature • Infant asymptomatic status • Epidural anesthesia • Diabetes • Illegal drug use • Preeclampsia • Lowest for age (ANC) • Gestation age • Birth weight • Parity • Antibiotic treatments • Placental problems • Meconium stained amniotic fluid • Cesarean section • Oligohidramnion
Makhoul et al., 2002 **VLBW** • Gender • Birth weight • Gestational age • Delivery room intubation • RDS • Mechanical ventilation • PDA • IVH • NEC • BPD	**Stoll et al., 2002** **3 days of age in VLBW** • Central venous catheter • Peripheral arterial line • Umbilical catheter • Hyperalimentation • Age at first feeding • Age at full feeding • Days to regain birth weight • Mechanical ventilation

<table>
<tr>
<td>Evans et al., 2007
neonatal mortality

Significant
• Sex
• Gestational age
• Weight for gestational age
• Apgar score
• Maternal ethnicity
• Baby presentation
• Transferred after birth
• Hypertensive disease in pregnancy
• Fetal distress
Non Significant
• Previous perinatal death
• Previous preterm baby
• Premature ROM
• Prolonged premature ROM
• Preterm labour
• Antepartum haemorrhage
• No antenatal steroids
• Singleton
• Maternal age
• Mode of delivery</td>
<td>Newton, 2005
infection related preterm birth

Historical
• Idiopathic preterm labor, PRM, or incompetent cervix
• History of UTI
• History of STI within current relationship
• History of STI during current pregnancy
• STI within previous 5 years
Behavioural
• Unintended pregnancy
• Unmarried
• New partner within 12 months
• Alcohol or drug abuse
• Uncertainty about partner fidelity
• Multiple current partners
Signs and symptoms
• Vaginal discharge
• Dysuria
• Dyspareunia
• Genital warts/dysplasia
• Genital ulcers
• Partners with genitourinary symptoms
• Asymptomatic bacteriuria</td>
</tr>
<tr>
<td colspan="2">Zaidi et al., 2005 - all neonates (hospital acquired)

• Lack of essential equipment and supplies (soap, wash basin, clean water, obstetric instruments, gloves, sterilizers, medications, cord clamps)
• Failures in sterilization/disinfection or handling/storage of multi-use resuscitation instruments, equipment and supplies, delivery surfaces, leading to contamination
• Reuse of disposable supplies without safe disinfection/sterilization procedures
• Inadequate hand hygiene and glove use
• Excessive vaginal examinations
• Lack of aseptic technique for invasive procedures and cord cutting and care
• Overcrowded and understaffed labour and delivery rooms
• Lack of knowledge, training, and competency regarding infection control practice and identification and management of risk factors for maternal and neonatal infection</td>
</tr>
</table>

Martius et al., 1999 **early onset below 35 weeks**	**Moore et al., 2005**
Maternal • Maternal leukocytosis • Maternal CRP • Maternal fever >38°C • PROM • Maternal GBS • Maternal antibiotics • Vaginal delivery • Funisitis and or chorioamnionitis Neonatal • Apgar score • Intracranial hemorrhage	Hospital acquired • Contaminated glucose-containing intravenous fluids. • Poor hand hygiene • Contaminated tap water • Contaminated ultrasound coupling gel • Inadequate sterilization of rectal thermometers • Inadequate aseptic techniques during preparation of intravenous fluids or medications

APPENDIX D

VARIABLES OF PREDICTIVE MODEL FOR NEONATAL SEPSIS AND NEONATAL ILLNESS SEVERITY SCORING SYSTEMS

1. GENERAL NEONATAL MORTALITY AND MORBIDITY

NICHHD Score (1993) **National Institute of Child Health and Human Development** • Birth weight • SGA • Race • Sex • Apgar score at 1 min	**NMPI (2000)** **Neonatal Mortality Prognosis Index** • Gestational age • Birth weight • Cardiac arrest • PaO_2/FiO_2 ratio • Major congenital malformations • Sepsis • Base excess
SINKIN 12 hour • Birth weight • Gestational age • Apgar score at 5 min • Peak inspiratory pressure at 12 h	**NBRS (1991)** **Nursery Neurobiologic Risk Score** • Blood pH • Hypoglycemia • IVH • Periventricular leukomalacia • Seizures • Infection • Need for mechanical ventilation
CRIB (1993) **Clinical Risk Index for Babies** • Gestational age • Birth weight • Base excess • Congenital malformations • Minimum appropriate FiO_2 • Maximum appropriate FiO_2	**CRIB II (2003)** **Clinical Risk Index for Babies** • Gender • Gestational age • Birth weight • Body temperature • Base excess

SNAP (1990)
Score for Neonatal Acute Physiology

- Blood pressure
- Heart rate
- Respiratory rate
- Temperature
- PO_2
- PO_2/FiO_2 ratio
- PCO_2
- Oxygenation index
- Packed cell volume
- WBC count
- Immature total ratio
- Absolute netrophil count
- Platelet count
- Blood urea nitrogen
- Creatinine
- Urine output
- Indirect bilirubin
- Direct bilirubin
- Sodium
- Potassium
- Ionized calcium
- Total calcium
- Glucose
- Serum bicarbonate
- Serum pH
- Seizure
- Apnea
- Stool guaiac

SNAPPE (1993)
Score for Neonatal Acute Physiology-Perinatal Extension

- SNAP score
- Birth weight
- Apgar score <7 at 5 min
- Small for date (<3 percentile birth weight)

SNAP II (2001)

- Body temperature
- PO_2/FiO_2 ratio
- Blood pressure
- Lowest serum pH
- Multiple seizures
- Urine output

SNAPPE II (2001)

- SNAP II score
- Birth weight $\leq$749 g
- Apgar <7 at 5 min
- Small for gestational age

Berlin Score (1997)

- Birth weight
- Grade of RDS
- Apgar score at 5 min
- Artificial ventilation
- Base excess at admission

MAIN (2005)
Morbidity Assessment Index for Newborns

- 11 variables within 11 hours
- 36 variables within 7 days of birth

NTISS (1992)
National Therapeutic Intervention Scoring System

- 59 variables, purely on management aspect

2. SPECIFIC SCORES FOR NEONATAL SEPSIS

Okascharoen et al, 2005 **late onset sepsis (72 hrs)** Clinical Variables : • Hypotension • Abnormal body temperature (fever, hypothermia, or temperature instability) • Respiratory insufficiency (apnea/bradycardia/tachypnea/cyanosis/ increased oxygen requirement or ventilator settings) Laboratory findings : • Neutrophil bandemia (band form fraction $\geq$1%) • Thrombocytopenia Management variables : • Presence of umbilical venous catheter	**Singh et al., 2003** **late onset sepsis** • Hyperthermia • Tachycardia • Abdominal distention • Increased pre-feed gastric aspirates • Grunting • Chest retractions • Lethargy
NOSEP (2000) **Mahieu et al., 2000 (nosocomial)** • CRP >10 mg/l • Neutrophil >50% • Thrombocytopenia <150 000/mm^3 • TPN duration $\geq$14 days • Fever >38.2^oC	**CLINICAL SCORING SYSTEM (2005)** **Dalgic et al., 2005 (nosocomial)** • Respiratory symptom • Abdominal distention • Feeding intolerance • Hypotension • Bradycardia • Lowest and highest body temperature difference
TOPS (2007) **Mathur and Arora, 2007** • Temperature • Oxygenation • Capillary refill time • Blood sugar	**Phillip and Hewitt (1980)** • Band/total neutrophil • Leucocyte count • CRP • ESR • Latex haptoglobin

Rodwell et al. (1988)	**Singh et al. (1994)**
• Abnormal total leucocyte count • Abnormal PMN count • Elevated immature PMN count • Elevated immature to total PMN ratio • Immature to mature PMN ratio • Platele count • Pronounced degenerative changes in PMNs	• Premature rupture of the membranes • Unclean vaginal examinations • Maternal pyrexia • Birth asphyxia • Prolonged labour • Foul smelling • Low birth weight
St Geme et al. (1984)	
• Placental inflammation • Gestational age • Sex – male • Apgar score 5 minutes • Clinical chorioamnionitis	•

Source : Phillip et al., 1980; St Geme et al., 1984; Rodwell et al., 1988; Singh et al., 1994; Mahieu et al., 2000; Singh et al., 2003; De Felice et al., 2005; Dorling et al, 2005; Okascharoen et al, 2005; Verma et al., 2005; Dalgic et al. 2005; Mathur and Arora, 2007

APPENDIX E

LIST OF INDEPENDENT VARIABLES, OPERATIONAL DEFINITIONS AND EXPLANATIONS OF VARIABLES

Table 49. List Of Initial Variables (I)

NO	VARIABLES	UNIT	CATEGORIES
	RISK FACTORS		
1	Prematurity	Weeks	Preterm/term
2	Length of hospital stay before sepsis	Days	
3	Length of hospital stay before sepsis - qualitative	Days	0-3/4-7/8-14/15-28
4	Sex		Male/female
5	Referred from		Rajvithi/other hospital/other
6	Frequency of antenatal care	Times	
7	Location of antenatal care		Hospital/clinic/other/none
8	Race		Thai/non thai/mixed
9	Mother age	Years	
10	Mother's age - qualitative	Years	1-20/21-30/31-35/36-55
11	Smoking		Yes/no
12	Alcohol		Yes/no
13	Genital disease		Yes/no
14	Chronic disease of mother		Yes/no
15	High risk pregnancy		Yes/no
16	Long term drug use		Yes/no
17	Education		None/elementary/high school/university
18	Occupation		None/office/shop/labor/government
19	Environment		Slum/factory/market/others
20	Number of pregnancy		
21	Number of delivery		
22	Number of abortion		
23	Space from previous child	Years	
24	Space from previous child – qualitative	Years	0/1-2/3-5/6<
25	Mode of delivery		Spontaneous/breech/CS/vacuum/forceps
26	Mode of delivery (operation/not)		Operation/not
27	Mode of delivery (spontaneous/not)		Spontaneous/not
28	Premature rupture of membrane		Yes/no
29	Chorioamnionitis		Yes/no
30	Smelly amniotic fluid		Yes/no
31	Color of amniotic fluid		Colorless/turbid/green/other
32	Fever of mother		Yes/no
33	Complication of pregnancy		None/APB/eclampsia/inf/plac prev/other
34	Location of birth		Hospital/home/other
35	Birth helper		Doctor/midwife/nurse/traditional/other
36	Steroid treatment before birth		Yes/no
37	Antibiotics treatment before birth		Yes/no
38	Singleton or twins		Singleton/twins
39	Birth weight	Grams	
40	Birth weight – qualitative	Grams	Normal/lbw/vlbw/elbw/4000<

Table 50. LIST OF INITIAL VARIABLES (II)

NO	VARIABLES	UNIT	CATEGORIES
	RISK FACTORS (cont'd)		
41	Gestational age	Weeks	
42	Gestational age - qualitative	Weeks	20-27/28-36/37-41/41<
43	Apgar 1 minute		
44	Apgar 1 - qualitative		0-3/4-6/7-10
45	Apgar 5 minute		
46	Apgar 5 - qualitative		0-3/4-6/7-10
47	Lubchenko		AGA/SGA/LGA
48	Intracranial hemorrhage		Yes/no
49	Umbilical cord		Fresh/not
50	Mode of resuscitation (yes/no)		Resuscitation/no
51	Mode of resuscitation		Mask/intubation/none
52	Seconds of resuscitation	Seconds	
53	Length of resuscitation – qualitative	Seconds	0/1-20/21-60/61-300/300<
54	Taking care by whom		Medical/family/other
55	Rearing after birth		Hospital/home/other
56	Cord treatment		Alcohol-t dye-iodine/none/other
57	Icterus after birth		Yes/no
58	Seizure		Yes/no
59	Fever of the baby		Yes/no
60	Breastfed		Yes/no
	CLINICAL CONDITION		
61	Lethargy		Yes/no
62	Poorfeeding		Yes/no
63	Cyanosis		Yes/no
64	Icterus in hospitalization		Yes/no
65	Systolic blood pressure	mmHg	
66	Diastolic blood pressure	mmHg	
67	Mean arterial pressure	mmHg	
68	Hypotension		Yes/no
69	Abnormal heart rate	x/min	Yes/no
70	Abnormal temperature	^{o}C	Yes/no
71	Mechanical ventilation		Yes/no
72	Respiratory pattern		Shallow/deep
73	Apnea episode		Yes/no
74	Grunting		Yes/no
75	Chest retraction		Yes/no
76	Respiratory insufficiency		Yes/no
77	Seizure in hospital		Yes/no
78	Capillary refill (2)	Seconds	0-3/3<
79	Urine production	cc/kg/hour	
80	Oxygen saturation	%	
81	Hypoxemia	%	Yes/no

Table 51. List of Initial Variables (III)

NO	VARIABLES	UNIT	CATEGORIES
	LABORATORY		
82	Hemoglobin	g/dl	
83	Anemia		Yes/no
84	Leucopenia	/cmm	Yes/no
85	Abnormal leukocytes	/cmm	Yes/no
86	Thrombocytes	/cmm	
87	Thrombocytopenia	/cmm	Yes/no
88	Band form	%	
89	Percentage of neutrophils	%	
90	Absolute neutrophils	/cmm	
91	I : T ratio (band : total neutrophil)		
92	Erythrocyte sedimentation rate	mm/hour	
93	Arterial blood pH		
94	Abnormal arterial pH		Yes/no
95	Arterial pO_2	mmHg	
96	Abnormal base excess		Yes/no
97	Abnormal potassium	mmol/l	Yes/no
98	Abnormal sodium	mmol/l	Yes/no
99	Abnormal calcium	mmol/l	Yes/no
100	Blood sugar	mg/dl	Yes/no
101	Hypoglycemia	mg/dl	Yes/no
102	Hyperglycemia	mg/dl	Yes/no
103	Hyperglycemia (> 180)	mg/dl	Yes/no
104	Abnormal glucose	mg/dl	Yes/no
105	Abnormal glucose (> 180)	mg/dl	
106	Blood urea nitrogen	mg/dl	Yes/no
107	Abnormal BUN	mg/dl	
108	Serum creatinine	mg/dl	Yes/no
109	Abnormal serum creatinine	mg/dl	Yes/no
110	Abnormal RFT		
111	AST	u/l	Yes/no
112	Abnormal AST	u/l	
113	ALT	u/l	Yes/no
114	Abnormal ALT	u/l	Yes/no
115	Abnormal LFT		
116	Direct bilirubin	mg/dl	Yes/no
117	Abnormal direct bilirubin (>0.2)	mg/dl	Yes/no
118	Abnormal direct bilirubin (>2)	mg/dl	
119	Total bilirubin	mg/dl	Yes/no
120	Abnormal total bilirubin	mg/dl	
121	CRP1	mg/dl	
122	CRP2 (the next 1-2 days)	mg/dl	Yes/no

Table 52. List of Initial Variables (IV)

NO	VARIABLES	UNIT	CATEGORIES
	LABORATORY (cont'd)		
123	CSF turbidity		
124	CSF pandy		0/1/2/3
125	CSF pandy - qualitative		Normal/abnormal
126	CSF color		Colorless/yellow/red
127	CSF color - qualitative		Colorless/with color
128	CSF opening pressure	cmH_2O	
129	CSF closing pressure	cmH_2O	
130	CSF WBC	cell/ul	
131	CSF WBC - qualitative	cell/ul	Normal/abnormal
132	CSF glucose	mg/dl	
133	CSF glucose - qualitative	mg/dl	Normal/abnormal
134	Percentage CSF glucose: blood glucose	%	
135	Percentage CSF glucose: blood glucose - qualitative	%	Normal/abnormal
136	CSF protein	mg/dl	
137	CSF protein - qualitative	mg/dl	Normal/abnormal
138	Urine protein	mg/dl	
139	Urine glucose		
140	Urine sediment WBC	/hpf	
141	Urine sediment RBC	/hpf	
142	Urine cast		
	TREATMENT		
143	Vascular catheter usage		Yes/no
144	Total parenteral nutrition		Yes/no

OPERATIONAL DEFINITIONS AND THE EXPLANATION OF VARIABLES

Diagnosis of Sepsis :

Follow the final diagnosis in the medical record and supported by the result of culture or other prove. Diagnosis without the laboratory result was not accepted.

Culture :

The cultures counted in this study were those originated from sterile sites only. The culture from urine and stool were not included. Culture results will be evaluated from the medical records, including classification as contaminant.

Sepsis group :

Sepsis group will be divided into 2 sub groups. The definitions are based on modification from the original definition mentioned previously. The modification is

concerning “prove of bacterial presence”. We add staining / latex / antigen-antibody test on this criteria.

- (Proven + Probable) group : clinical + culture positive / PCR / Latex / Antigen-Antibody test / Staining
- Possible group : based only on clinical signs and non-specific laboratory examinations

The possible group will not be included in this study.

Sepsis episodes :

Sepsis patients will be counted per episode. It means if 1 patient has 2 episodes of sepsis during hospitalization, those 2 episodes will be counted separately as long as the age of the patients at the time of sepsis diagnosis not more than 28 days.

Non-bacterial sepsis :

Only bacterial sepsis will be included. Non bacterial sepsis, as shown in the culture result, will be excluded.

Early sepsis :

Early sepsis is sepsis $\leq$ 7 days.

Very early sepsis :

Sepsis diagnosed on the first day of life

Late sepsis :

Late sepsis is sepsis diagnosed >7 days of life. Late late sepsis (or sepsis diagnosed >30 days of life) is not included in this study.

Neonates :

Infants with the age of $\leq$ 28 days

Low Birth Weight (LBW) :

Neonates less than 2500 grams

Very Low Birth Weight (VLBW) :

Neonates with the body weight of 1500 grams or less

Extremely Low Birth Weight (ELBW) :

Neonates with the body weight of1000 grams or less

Preterm :

Less than 37 weeks of gestation counted from the onset of the last menstrual period as per the history given by the mother and recorded in the medical record of the neonates.

Severe congenital malformation :

All neonates with severe congenital malformation such as severe congenital heart disease, severe hydrocephalus, and gastroschizis, or malformations which are not compatible with life will be excluded.

Surgery :

Neonates undergo surgery before the diagnosis of sepsis will be excluded. If the surgery has not been done yet the patient still can be included

Length of stay before sepsis :

For case group it was counted from the date of admission until the date of sepsis. For control group it was counted from the date of admission until the date when data were taken.

Race :

In this study the race will be divided into 3 : thai, non thai, and mixed.

High Risk Pregnancy :

This will include preeclampsia, antepartum bleeding, malpresentation of the baby, and other conditions as decided by the doctor of the mother. Diagnosis of high risk pregnancy will be based on the referral letter or medical record of the mother

Long Term Drug :

Mother consumes the same drug more than 1 month. Data about long term drug will be based on the referral letter or medical record of the mother

Chorioamnionitis :

Diagnosis of chorioamnionitis will be based on the referral letter from the previous doctor or medical record of the mother. Either clinical or histopathological based diagnosis will be accepted.

Maternal fever :

It meant core temperature of $\geq 38^{o}C$ before delivery. This data will be based on the record of the mother.

Premature rupture of the membranes :

Rupture of membranes ≥ 18 hours before delivery (Leaking of amniotic fluid before the onset of labor)

Resuscitation :

It meant resuscitation immediately after delivery

Neonatal fever :

Core temperature ≥38^{o}C

Breastfed :

It can be exclusively breastfed or partially breastfed.

Clinical manifestations :

Data about clinical manifestations will be taken from the worst data of the last 24 hours before diagnosis of sepsis made. If such data are not available, the nearest previous data will be used.

Laboratory results :

Data about laboratory results will be taken from the worst data of the last 24 hours before or – for the second choice - 24 hours after diagnosis of sepsis made. If such data are not available, the nearest previous data will be used.

Lethargy :

Decrease responsiveness to external stimuli, coma, or hypotonia.

Poor feeding :

Having any difficulty in feeding, including vomiting, feeding intolerance, and left too much retention when the gastric tube aspirated.

Mean arterial pressure :

It was counted by : ⅓ (lowest systolic + lowest diastolic).

Hypothermia :

Core body temperature <36.5^{o}C

Respiratory insufficiency :

At least one of the following conditions was found : apnea, tachypnea, grunting, and or chest retraction.

Hyperglycemia :

Blood glucose > 140 mg/dl

Hypoglycemia :

Blood glucose <40 mg/dl

CRP :

Considered as positive if >10 mg/dl

Blood Components :

All kinds of blood components will be included

Vascular catheter :

Included umbilical, venous - peripheral, and central catheters

Risk Factors :

Most risk factors known from the previous studies will be included in the analysis.

Diagnostic method :

Best combination of several variables/parameters in the independent variables group

Dependent variable :

In the main analysis, dependent variable is sepsis or non sepsis

Independent variable :

In the main analysis independent variables are collected from risk factors, clinical features, laboratory results, and treatment modalities from several published studies, including some scoring system in neonatology.

Season :

This study will use month of birth as one variable. There were 3 seasons in Thailand namely rainy (June-September), winter (October-January), and summer (February-May).

The term "qualitative" :

The continuous data were transformed to qualitative form and considered as other variable. To distinguish from the original continuous data, the word "qualitative" was added.

The term "abnormal" :

If the variable had 2 possibilities, lowest and highest, then the value out of this range was considered as "abnormal"

Variables with more than 1 choice of reference value :

Since variables could be treated differently with more than 1 choices of reference value, the possibilities were added as new variables. Three variables – leucocytes, blood glucose and direct bilirubin – used this approach. Tables below show the reference values and cutoff points.

Coding :

For dichotomous variables, “0” was used for normal condition and “1” for abnormal condition. For more than 2 categories variables, the higher numbers were used.

APPENDIX F

NORMAL VALUE FOR REPLACING MISSING DATA AND CUT OFF VALUE FOR VARIABLES

Table 53. Normal Value Used for Replacing Missing Data – Clinical Conditions and Complete Blood Count

VARIABLES	AGE	VALUE	NOTE	SOURCE
CLINICAL : RESPIRATION AND URINE PRODUCTION				
• Highest respiration rate (x/min)	0-28 days	Early 59 Late 55	30-60	
• Lowest respiration rate (x/min)	0-28 days	Early 45 Late 44		
• Urine production (cc/kg/hour)	0-28 days	1	>0.5	4
COMPLETE BLOOD COUNT				
• Hemoglobin (g/dl)	Term-<2wk	16.5	13.5-19.5	1,2
	Term-≥2wk	16.5	12.1-18.2	1,2
	Preterm	16.1	13.2-18.8	2
• Leucocyte (/cmm)	Birth	18 100	Mean	1
	0-12 hr	22 800		
	12-24 hr	18 900		
	1-7 day	12 200		
	8-14 day	11 400		
	15-28 day	10 800		
• Thrombocyte (/cmm)	Term-<3 day	192 000	Mean	1
	Term-3-7 day	248 000		
• Percentage neutrophil (%)	Term-1 day	61	Mean	1
	Term-1 day	11 500		
• Absolute neutrophil (/cmm)	Term-2-7 day	5500	Mean	1

Table 54. Normal Value Used for Replacing Missing Data – Blood Gas Analysis, Electrolytes, Blood Sugar, RFT, And LFT

VARIABLES	AGE	VALUE	NOTE	SOURCE
BLOOD GAS ANALYSIS AND ELECTROLYTES				
• pH		7.4	7.27-7.45	2
• Base excess		0	(-5) – (+5)	4
• Oxygen saturation		95	≥92	4
• Potassium	Term	5.2	4.4-6.4	2
	Preterm-1 wk	5.6	4.6-6.7	2,3
	Preterm-3 wk	5.8	4.5-7.1	2,3
• Sodium	Term	137	130-142	2
	Preterm-1 wk	139.6	133-146	2,3
	Preterm-3 wk	136.3	129-142	2,3
• Calcium (mg/dl / mEq/l)	Term	8.2 / 2.6	7.9-8.4 / 2.38-2.80	3
	Preterm-1 wk	9.2 / 2	6.1-11.6 / 1.75-3.0	1,2,3
	Preterm-3 wk	9.6 / 2.2	8.1-11 /	2,3
BLOOD SUGAR, RFT, AND LFT				
• Blood sugar (mg/dl)	Term-1 day	50	40-60	1
	Term->1 day	65	50-80	1
	Preterm	40	20-60	1
• BUN (mg/dl)	Term	7.1	4-12	1,2
	Preterm-1 wk	9.3	3.1-25.5	2,3
	Preterm-3 wk	13.3	2.1-31.4	2,3
• Serum creatinine (mg/dl)	Term-1-2 day	0.7	Mean	2
	Term-1 wk	0.5	Mean	2
	Term-3 wk	0.3	Mean	2
	≤2500g-2 day	1	0.9-1.1	2
	<2500g-1 wk	0.6	0.5-0.7	2
	<2500g-3 wk	0.5	0.4-0.6	2
• AST (U/L)		50	25-75	1
• ALT (U/L)		26	7-46	1
• Direct bilirubin (mg/dl)		0.1	0.0-0.2	1
• Total bilirubin (mg/dl)	Term-1 day	4	0-8	1
	Term-2-28 d	6	0-12	1
	Preterm-1 day	4	0-8	1
	Preterm-2 day	6	0-12	1
	Preterm->2 d	8	0-16	1

Table 55. Normal Value Used for Replacing Missing Data – Cerebrospinal Fluid

VARIABLES	AGE	VALUE	NOTE	SOURCE
CEREBROSPINAL FLUID				
• Colour		Clear or xantochrome		2
• WBC (/hpf)	Preterm	13	0-25	1
	Term	11	0-22	1
• Glucose (mg/dl)	Preterm	44	24-63	1
	Term	77	34-119	1
• Protein (mg/dl)	Preterm	108	65-150	1
	Term	95	20-170	1

Source : Barone 2006 (1)
Nock and Patra, 2006 (2)
Gleason 2005 (3)
Goldstein et al., 2005 (4)

Table 56. Cutoff Value for Laboratory Data – Complete Blood Count

NO	VARIABLES
1	HEMOGLOBIN (1,2) Term < 2 weeks : 13.5-19.5 mg/dl Term ≥ 2 weeks : 12.1-18.2 mg/dl Preterm : 13.2-18.8 mg/dl MANROE'S CRITERIA FOR WHITE BLOOD CELL COUNT (Manroe's) Normal WBC : < 7 days of age : 9000 – 30 000 /cmm 7-14 days of age : 5000 – 21 000 /cmm > 14 days of age : 5000 – 20 000 /cmm Normal PMN : < 7 days of age : 6000 – 26 000 /cmm 7-14 days of age : 1500 – 10 000 /cmm > 14 days of age : 1000 – 9500 /cmm

Table 57. Cutoff Value for Laboratory Data – Electrolytes, RFT, LFT, and Cerebrospinal Fluid

NO	VARIABLES
2	Potassium (2,3) Term : 4.4-6.4 mmol/l Preterm 1 week : 4.6-6.7 mmol/l Preterm 3 weeks : 4.5-7.1 mmol/l Sodium (2,3) Term : 130-142 mmol/l Preterm 1 week : 133-146 mmol/l Preterm 3 weeks : 129-142 mmol/l Calcium (2,3) Non-ionized : Term 2.38 – 2.80 mEq/l Preterm 1.75 – 3 mEq/l Ionized : Term 0.9 – 1.3 mmol/l Preterm 1.05 – 1.56 mmol/l
3	RENAL FUNCTION TESTS BUN : Term : 4-12 mg/dl Preterm 1 week : 3.1-25.5 mg/dl Preterm 3 weeks : 2.1-31.4 mg/dl Serum Creatinine (2,6) : Term : <1.0 mg/dl Preterm 2 days : < 1.5 mg/dl Preterm 1 week : < 1 mg/dl
4	LIVER FUNCTION TESTS Direct Bilirubin (1) (a) : < 0.2 mg/dl (b) : < 2 mg/dl Total Bilirubin (1) : Term 1 day : 0-8 mg/dl Term 2-28 days : 0-12 mg/dl Preterm 1 day : 0-8 mg/dl Preterm 2 days : 0-12 mg/dl Preterm > 2 days : 0-16 mg/dl
5	CSF Percentage csf glucose : blood glucose : ≥ 60%

APPENDIX G

RESULT OF SPSS

MULTIPLE LOGISTIC REGRESSION (ENTER)

EARLY SEPSIS

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
								Lower	Upper
Step 1(a)	LOSBEFQ	2.364	.932	6.431	1	.011	10.634	1.711	66.097
	POORFEE	1.268	.489	6.720	1	.010	3.553	1.362	9.265
	LEUKOPEN	1.872	.763	6.015	1	.014	6.499	1.456	28.999
	THROMBQ	1.540	.520	8.765	1	.003	4.664	1.683	12.927
	ABNO2SQ	1.896	.535	12.540	1	.000	6.660	2.332	19.022
	Constant	-2.895	.386	56.226	1	.000	.055		

a Variable(s) entered on step 1: LOSBEFQ, POORFEE, LEUKOPEN, THROMBQ, ABNO2SQ.

Classification Table(a)

	Observed		Predicted		
			case sepsis group		Percentage Correct
			control	sepsis	
Step 1	case sepsis group	control	124	11	91.9
		sepsis	17	28	62.2
	Overall Percentage				84.4

a The cut value is .500

LATE SEPSIS

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
								Lower	Upper
Step 1(a)	POORFEE	2.398	.630	14.498	1	.000	10.996	3.201	37.776
	ABNHRQ	3.087	1.165	7.023	1	.008	21.920	2.235	215.020
	ABNTEMQ	3.995	1.046	14.595	1	.000	54.334	6.997	421.915
	ABNLEUQ	1.786	.835	4.578	1	.032	5.967	1.162	30.645
	ABNPHQ	2.479	.614	16.321	1	.000	11.924	3.583	39.690
	ABNO2SQ	1.387	.698	3.949	1	.047	4.004	1.019	15.729
	Constant	-4.328	.612	50.039	1	.000	.013		

a Variable(s) entered on step 1: POORFEE, ABNHRQ, ABNTEMQ, ABNLEUQ, ABNPHQ, ABNO2SQ.

Classification Table(a)

Observed			Predicted		
			case sepsis group		Percentage Correct
			control	sepsis	
Step 1	case sepsis group	control	150	6	96.2
		sepsis	13	39	75.0
	Overall Percentage				90.9

a The cut value is .500

APPENDIX H
STATEMENT ABOUT PUBLICATIONS

Publications of this study will follow rules in Faculty of Tropical Medicine Mahidol University, Faculty of Graduate Studies Mahidol University, and Queen Sirikit National Institute of Child Health, including all necessary official permissions.

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