Thrombocytopenia as A Predictor of Neonatal Sepsis in Very Low Birth Weight Babies and Its Correlation with Specific Organism Involved: A Hospital Based Observational Study

Parvez Ahmad, Rajnesh Kaith, Imran Gattoo, Bilal Ahmad Najar, Sheikh Quyoom Hussain

ABSTRACT

Introduction: Thrombocytopenia is one of the common haematological problems encountered in the neonatal period. During an underlying pathologic process, the sick newborns, premature babies and neonates admitted in neonatal intensive care units are usually predisposed to develop thrombocytopenia.

Aims: To study the bacteriological profile and thrombocytopenia as a predictor of neonatal sepsis in very low birth weight neonates and its correlation with specific organism involved.

Materials and Methods: This study is a prospective observational study conducted from May 2012 to April 2013. The study group included VLBW neonates admitted as neonatal sepsis. Sepsis evaluation was based on clinical signs and symptoms and rapid tests for sepsis, including change in complete blood counts and positive blood culture. The platelet parameters studied include total platelet count, durations of thrombocytopenia, change in platelet count and platelet nadir.

Results: Total of 100 newborn very low birth babies with culture documented sepsis was studied. 54(54%) had gram negative sepsis, 40(40%) had gram positive sepsis and 6(6%) has fungal sepsis. Among gram negative organisms, Klebsiella pneumonia was seen in 26(48.1%), followed by Pseudomonas 10 (18.5%) and Acinetobacter 8 (14.8%). Among gram positive organism MRSA was the commonest 21 (52.5%) followed by Enterococcus 12/40(30.0%) and CONS 4 (10.0%). Severe thrombocytopenia (count less than 50000/µl) were present in 22.2% in gram negative, 2.5 % in gram positive organism and 33.3% in fungal sepsis.(p=0.02). Mean platelet count in survivors change from 142×10^3/µl to 94.6×10^3/µl at onset of sepsis in survivors while in non survivors it changes from 150×10^3/µl to 80.2×10^3/µl within 48 hours of admission.

Conclusion: Gram negative sepsis was predominant cause of sepsis followed by Gram positive organisms and fungal sepsis. Among gram negative organisms Klebsiella pneumonia predominated followed by Pseudomonas and Acinetobacter. Among gram positive MRSA was the commonest followed by Enterococcus and Coagulase negative Staphylococci. Both Severe thrombocytopenia and most significant fall in the platelet count was seen more in fungal sepsis than in bacterial sepsis. There was significant fall in platelet count in non survivors than those who survived.

INTRODUCTION

Throughout the world, more than half a million newborns are estimated to die from serious neonatal infections [1]. Although most of these deaths are in developing countries, where neonatal mortality from sepsis may be as high as 60% [2]. The incidence of infection in developed world is also very high at 2.2 to 8.6 per 1000 live births [3]. The importance of the neonatal mortality can be understood by the fact that half of deaths of children less than 5 years of age and two-thirds of infant deaths are contributed by neonatal deaths only [4]. Neonatal septicaemia continues to be an important cause of morbidity and mortality in neonates especially in VLBW [5]. Decreased function of neutrophils and other cells involved in response to infection have been demonstrated in both term and preterm infants. Preterm infants also have low concentration of immunoglobulin [6]. According to International sepsis definition conference...
sepsis is defined as clinical syndrome characterized by presence of both infection and systemic inflammatory response syndrome (SIRS). Systemic inflammatory response syndrome in case of neonates is characterized by two or more of following:

1. Tachypnea [respiratory rate  ≥ 60 breaths per minutes (bpm)] plus grunting or retractions or desaturation.
2. Temperature instability (<36°C or > 37.9°C)
3. Capillary refill time ≥ 3 sec.
4. White blood cell count (<5000/µl or > 34,000/µl)
5. CRP 10mg/dl or 2 SD above normal value
6. Interleukin 6 or 8 ≥70 pg/ml
7. Procalcitonin 8.1mg/dl or 2 SD above normal value.

Sepsis is defined as one or more systemic inflammatory syndrome criteria with signs of infection [9]. Severe sepsis is defined as sepsis accompanied by single organ dysfunction, hypo perfusion or hypotension. Septic shock ensues when hypotension and poor organ perfusion develops despite adequate fluid replacement [10]. Finally in presence of altered organ function in an acutely ill patient, to the extent that homeostasis cannot be maintained without intervention multiple organ dysfunction is diagnosed [10].

In neonates, sepsis is usually associated with isolation of an organism from either blood, cerebrospinal fluid (CSF), or urine, associated with systemic inflammatory response syndrome [11]. Sepsis accounts for approximately half of all deaths beyond second week of life in VLBW babies [12].

Incidence of neonatal sepsis varies from 1-4 cases per 1000 live births in developed countries [6]. In fact, 48% of all infections during childhood occur in children of <1 year of age, and just over half (27%) of these occur in newborn period. Between 33-66% of babies admitted in neonatal intensive care units are diagnosed to have infections some time during their stay in neonatal units [3]. Term male infants have an approximately two fold higher incidence of sepsis than term females. This sex difference is less clear in preterm low birth weight (LBW) infants [6].

Prematurity and LBW are important predisposing factors for infection in neonatal period. Such infants have a 3 to 10 fold higher incidence of infection than full term normal birth weight infants. Preterm and VLBW babies are also predisposed to high rate of nosocomial infection [6]. Coagulase negative *staphylococci* are the most frequent neonatal nosocomial pathogens. According to National Institute of Child Health and Human Development (NICHD) research network gram positive agents cause sepsis in 70%, gram negative with 18% and fungi in 12% of cases of late onset sepsis (LOS) [6]. However in some reports from United States, gram negative organisms are increasingly being cultured [13]. Mortality in nosocomial infections is low in newborn, but recent evidence suggests that nosocomial infection may not merely prolong hospital stay but may also cause long term neurological morbidity [14].

Thus early diagnosis of sepsis in neonates is warranted to interrupt the cascade of events leading to septic shock and multiorgan failure.

Platelet count less than 150 x 10^9/µl in any neonate is defined as thrombocytopenia regardless of gestation age [15,16]. Thrombocytopenia is used as an early but non-specific marker of sepsis in neonates [17]. More than 30-80% of neonates with proven infection become thrombocytopenic [18]. Bacterial, fungal and viral infections all have been associated with neonatal thrombocytopenia [19]. Thrombocytopenia occurs in one-third of infants admitted in neonatal intensive care unit [20]. In these patients thrombocytopenia presents either as early onset (less than 72 hours) or late onset thrombocytopenia (more than 72 hours). Early onset thrombocytopenia is most commonly due to platelet insufficiency and is mild and self-limiting while late onset thrombocytopenia is commonly due to bacterial sepsis and necrotizing enterocolitis and is often severe and prolonged [21]. Usually there can be multiple causes of thrombocytopenia in septic neonates but broadly it can be due to increased platelet destruction, decreased platelet production or mixed etiology [22]. Platelets interact with invading microorganisms and are critically linked to proinflammatory innate immune response. Platelets express Toll Like Receptors (TLR) especially TLR-4 and this expression significantly modulates microbial lipopolysaccharide induced thrombocytopenia [23]. This explains the clinical observation of severe thrombocytopenia associated with sepsis [15]. Thus platelets may play an important role in host defence by acting as circulating pathogen sentinels to initially alert cells of innate immune system.

By the time sepsis is diagnosed 25% neonates have thrombocytopenia and by 36-48 hours later majority of neonates develop thrombocytopenia. Fungal sepsis is associated with greater degree of thrombocytopenia than is seen with either gram positive or gram negative bacterial organisms and outcome in these neonates is poor [24]. Keeping in view the paucity of literature regarding the organism specific platelet response in neonates with sepsis, and lack of similar regional study to the best of our knowledge, we conducted this study to know the incidence of thrombocytopenia in neonates with sepsis, and also the effect of different organisms on various platelet parameters in neonates.

**MATERIALS AND METHODS**

This study is a prospective observational study conducted from May 2012 to April 2013 in GB Pant
Hospital, a tertiary care referral Paediatric Hospital in Jammu and Kashmir India. Total of 100 VLBW babies with culture documented sepsis were included after taking consent from their parents/guardian. Babies with hospital acquired sepsis, congenital malformations and chromosomal anomalies were excluded from the study. Ethical clearance was given by the hospital ethical committee.

Sepsis evaluation was based on clinical signs and symptoms and rapid tests for sepsis, including change in complete blood counts and positive blood culture.

Under all aseptic conditions 1ml of blood was drawn out from one of the peripheral veins for the blood cell count and culture. Platelet counts were carried out on counter. The platelet parameters studied included total platelet count, durations of thrombocytopenia, change in platelet count and platelet nadir. Thrombocytopenia was defined as platelet counts less than 150x10^3/µl. The duration of thrombocytopenia was the number of continuous days during which the platelet remained less than 150x10^3/µl. Platelets nadir was the lowest platelet count obtained for that neonate starting from the periods in which the blood culture is drawn. Outcome in the form of mortality, multiorgan failure was analysed. Mortality was defined as death before discharge. Infants discharged to home are considered survivors. All the data was recorded in a pre-structured proforma and data was analysed as per standard statistical methods.

RESULTS
Total sample of 100 newborn very low birth babies with culture documented sepsis was taken. To study the bacteriological profile and thrombocytopenia as a predictor of neonatal sepsis in very low birth weight neonates [Table/Fig-1] shows average gestation age (in weeks) among different organism groups. [Table/Fig-2] mean birth weight (in kg) among different organism groups. 

<table>
<thead>
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<th>Author</th>
<th>n</th>
<th>Mean Gestational Age ± SD</th>
<th>p value</th>
</tr>
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<tr>
<td>Gram Negative</td>
<td>54</td>
<td>31.91 ± 1.916</td>
<td>0.360</td>
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<tr>
<td>Fungal</td>
<td>6</td>
<td>30.83 ± 1.602</td>
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</table>

[Table/Fig-1]: Showing mean gestational age (GA in weeks) among different organism groups

<table>
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<tr>
<th>Author</th>
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<th>Mean ± SD</th>
<th>p value</th>
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<tbody>
<tr>
<td>Gram Negative</td>
<td>54</td>
<td>1.261 ± 0.169</td>
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<tr>
<td>Gram Positive</td>
<td>54</td>
<td>1.232 ± 0.1953</td>
<td>0.731</td>
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<tr>
<td>Fungal</td>
<td>6</td>
<td>1.233 ± 0.1506</td>
<td></td>
</tr>
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[Table/Fig-2]: Showing mean birth weight (in kg) among different organism groups

Out of 100 premature rupture of membranes (PROM) was present in 18 (18%) cases. Out of these 11 (20.4%) babies with gram negative sepsis, 6 (15%) with gram positive sepsis and 1 (16.7%) fungal sepsis had PROM (p=0.796).

As depicted in [Table/Fig-4] there was no significant difference in gestational age, premature rupture of membranes, meconium stain, pulmonary hemorrhage and maternal fever among different groups of organisms. [Table/Fig-5] showing mean platelet count at onset of sepsis in gram positive organism decreases from 152x10^3/µl to 10^3/µl while in gram negative organism from 140x10^3/µl to 81.8x10^3/µl and in fungal sepsis it decreases from 149x10^3/µl to 73.9x10^3/µl (p<0.017).

Severe thrombocytopenia (count less than 50000/µl) were present in 22.2% in gram negative, 2.5 % in gram positive organism and 33.3% in fungal sepsis (p<0.02) [Table/Fig-6].

[Table/Fig-7] shows that mean platelet count change from 142x10^3/µl to 94.6x10^3/µl at onset of sepsis in survivors while in non survivors it changes from 150x10^3/µl to 80.2x10^3/µl.

DISCUSSION
Neonatal septicaemia requires rapid and accurate diagnosis along with prompt treatment for neonatal survival and neurodevelopment outcome. Since decreased platelet count is frequently encountered in sick, preterm and full term babies [25]. One of the most common haematological manifestation during early
sepsis is thrombocytopenia, thus platelet count may be act as an early marker for the diagnosis of septicaemia [26].

Most commonly it is the gram negative organisms responsible for neonatal sepsis. In our study of VLBW, 56% cases are due to gram negative organisms. Among gram negative, 26 cases were due to *Klebsiella pneumonia*. Similar trend has been reported from developing as well as some developed countries. Rehman et al., [27] reported gram negative organisms as the commonest cause of neonatal sepsis with *E. coli* (36%) accounting for most of cases. Khassawneh et al., [28] from Jordan also reported gram negative organisms as the commonest cause of neonatal sepsis.

In our study MRSA was the second most common organism responsible for 21% of all cases. Rehman et al., [27] also reported *Staphylococcus aureus* responsible for neonatal sepsis in 29% of patients, Kurein et al., [29], reported *Staphylococcus aureus* in 13% of cases with LOS. *Staphylococcus aureus* colonizes skin, nasopharynx and GIT and spreads via hands of health care workers [30]. This implies a need for better adherence to hygiene practices, cohorting and isolation and decolonization of health care workers.

Coagulase negative *Staphylococcus aureus* (CONS) was seen in 4% cases in our study. Venkateshan [30] had reported 5-6% incidence of CONS in late onset sepsis. In developed countries CONS is the major causative organism of late onset sepsis. Sanghvi in 1996 [31] had reported CONS in 61% cases of late onset sepsis. CONS were isolated less commonly in our study.

In our study yeast was grown in 6 cases of sepsis. Venkateshan [30] had reported that 11% of septic...
neonates were having fungal sepsis. Guida et al.[32] had reported that 8% of septic neonates in their study were having fungal sepsis. In recent study (2009) Bhat et al., [9] reported that 8.5% of septic VLBW neonates were having fungal sepsis. T Calveros [33] in 2007, have reported incidence of fungal sepsis in 1% of VLBW neonates.

In our study, mean platelet count at the time of onset of sepsis was more in gram positive sepsis (103×10^9/µl) as compared to gram negative sepsis patients (81.8×10^9/µl) and fungal sepsis patients (73.9×10^9/µl) (p=0.017). Guida et al., [32] in 2003 reported significantly low platelet count at onset of sepsis in gram negative and fungal sepsis. Akarsu et al., [34] had shown lowest platelet count in gram negative as compared to gram positive sepsis.

Platelet nadir in our study was 20×10^9/µl in gram negative sepsis, 22×10^9/µl in fungal sepsis and 22×10^9/µl in gram positive sepsis. Guida et al., [32] in 2003 had similarly reported low platelet nadir in gram negative and fungal sepsis. Akarsu et al., [34] also reported lowest platelet count in gram negative sepsis as compared to gram positive sepsis. Bhat et al., [9] 2009 had reported significantly low platelet nadir in gram negative and fungal sepsis as compared to gram positive sepsis.

The mean duration of thrombocytopenia in our study was 5.42 days in gram positive sepsis, 7.42 days in gram negative sepsis and 5.5 days in fungal sepsis. The difference between the groups was statistically significant (p=0.02). Similar findings were reported by Bhat et al., [9], in their study the average duration of thrombocytopenia was 2.5 days in gram positive sepsis and 8.3 days in gram negative and fungal sepsis (statistically significant). Guida et al., [32] had reported the mean duration of thrombocytopenia of 0.4 days in gram positive sepsis and 2 days in gram negative and fungal sepsis. The greater duration in gram negative sepsis may be attributed to the fact that gram negative sepsis causes severe thrombocytopenia. Thrombocytopenia in gram negative and fungal sepsis induced is very severe and takes longer time to return to baseline [35].

Various platelet parameters are influenced in Klebsiella pneumonia sepsis. Virulence of Klebsiella pneumonia is determined by a smooth lipopolysaccharide (LPS with O antigen) and capsular polysaccharide (K antigen). Klebsiella pneumonia and other gram negative organisms have a variation in genetic makeup of O antigen, which allows Klebsiella pneumonia to resist, complement mediated opsonophagocytic killing [36].

In our study there was 35% (35 out of100) mortality. Among gram negative sepsis mortality was 46.29% (25) while gram positive had 20% (8) mortality and fungal sepsis had 33.3% (2) mortality. Study from Khassawneh et al., [28] had found similar high mortality in gram negative sepsis. Akarsu et al., [34] had shown more mortality in gram negative sepsis. In their study mortality in gram positive sepsis was 13% and in gram negative sepsis was 17% (p=0.05). Our study had similar results with statistically significant difference. Torkman et al., [37] had reported mortality of 15% in babies with sepsis. Khassawneh et al., [28] in 2009 had reported 31% mortality in neonatal sepsis from Jordan. Vankateshan et al., [30] had documented 42% mortality in neonatal sepsis in 1996 which gradually decreased to 20% in 2006.

While comparing the survivor and non survivor babies with thrombocytopenia we found that babies who survived had gestational age (32.26 weeks) than that of non-survivors (30.51weeks) (p=0.001). The average weight of survived babies was significantly more (1.302 kg) as compared to non survivors (1.147kg) (p≤0.001).

Although not much studies on platelet count in sepsis, yet it has well been described for more than 40 years that patients with sepsis often have thrombocytopenia and the intravenous injection of lipopolysaccharides in mice induce rapid thrombocytopenia [38] . All sepsis agents can cause thrombocytopenia in new born. Platelets are believed as active participants in host defence. Diffuse endothelial cell trauma, bacterial, fungal toxins, increased platelet activation and increased platelet consumption due to DIC are among the factors that play a role in the mechanism of thrombocytopenia. Lipopolysaccharide, which is a component of cell wall of gram negative organisms, leads to thrombocytopenia. Lipid – A, a component of lipopolysaccharide, increases the consumption.

Platelets play an important role in linking innate and adaptive immune responses [39]. Platelets have signalling receptors, known as Toll like Receptors (TLR) which helps in recognising bacteria, fungi and viruses. TLR play important role in stimulation of adaptive immunity against invading micro organisms.

**CONCLUSION**

Gram negative sepsis was predominant cause of sepsis followed by Gram positive organisms and fungal sepsis. Severe thrombocytopenia was more prevalent in fungal sepsis than in patients affected by gram negative and gram positive organisms. Thus thrombocytopenia can act as one of the earliest nonspecific indicator of neonatal sepsis and also correlates significantly with the outcome of the septic VLBW newborns. Small sample size is one of the limitations of the study; further studies are required with larger sample size to establish the role of thrombocytopenia in VLBW babies as a predictor of neonatal sepsis.

**ACKNOWLEDGMENT**

The authors want to thank the parents and the guardians who consented for the participation of their children in the study.
REFERENCES


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FINANCIAL OR OTHER COMPETING INTERESTS:
None.

Date of Publishing: Jul 01, 2015