# original research report

# Nosocomial sepsis-induced late onset thrombocytopenia in a neonatal tertiary care unit: a prospective study

Bashir Ahmad Charoo, Javeed Iqbal, Qazi Iqbal, Sheikh Mushtaq, Abdul Wahid Bhat, Imtiyaz Nawaz

From the Department of Pediatrics Sher-I-Kashmir Institute Of Medical Sciences Soura Srinagar Jammu and Kashmir, India

Correspondence: Bashir Ahmed Charoo, MD · Mominabad Noorbagh Sopore 193201· bash678@rediffmail.com · Accepted for publication June 2009

**BACKGROUND AND OBJECTIVES:** Late onset sepsis (LOS)( onset of sepsis >72 hours of age or nosocomial sepsis) is an important cause of morbidity and mortality in the neonatal intensive care unit (NICU). Thrombocytopenia is an important complication of sepsis. We investigated the incidence of thrombocytopenia in LOS patients and studied the influence of various parameters on platelet response.

**PATIENTS AND METHODS:** Infants born in the level 3 neonatal intensive care unit between January 2002 and December 2006 with documented LOS were included in this prospective study. Multiple hemograms with platelet counts, bacterial blood culture and fungal blood culture were obtained in all patients. Demographic and clinical data were compared between patients without thrombocytopenia and with mild, moderate and severe thrombocytopenia. Duration of thrombocytopenia in relation to type of organism and mortality with respect to degree of thrombocytopenia were also studied.

**RESULTS:** Of 200 patients with culture-proven nosocomial sepsis, 119 (59.5%) patients developed thrombocytopenia (platelet count <150×10$^9$/L). In our series *Klebsiella pneumoniae* was the most frequently isolated organism (125/200, 62.5%) and the incidence of thrombocytopenia was 60.0% (75/125). However, the incidence of thrombocytopenia was highest among patients who had concurrent bacterial and fungal sepsis (28/31, 90.3%). Coagulase-negative staphylococcal (CoNS) sepsis was present in 21 (10.5%) patients and the incidence of thrombocytopenia was 33.3%. Isolated fungal sepsis was present only in 6 (3%) patients and the incidence of thrombocytopenia was 66.0%. The incidence of thrombocytopenia was highest among preterm babies and low-birth weight (LBW) babies. Twenty-seven percent (54/200) of babies presented with mild thrombocytopenia, 20% (40/200) presented with moderate thrombocytopenia, and 12.5%(25/200) developed severe thrombocytopenia. Severity of thrombocytopenia was also directly related to the presence of necrotizing enterocolitis (NEC) and disseminated intravascular coagulation (DIC). The mortality rate was significantly associated with the degree of thrombocytopenia.

**CONCLUSION:** LOS sepsis is an important risk factor for thrombocytopenia in the NICU. Fungal and gram-negative sepsis are frequently associated with a decreased platelet count. Sepsis-induced thrombocytopenia is more common among LBW babies and preterm babies. The mortality rate is significantly related to degree of thrombocytopenia.

Early onset sepsis is defined as sepsis that occurs within the first 72 hours of birth. Late-onset sepsis (LOS)(onset of sepsis >72 hours of age or nosocomial sepsis) is an important cause of morbidity and mortality in the neonatal intensive care unit (NICU)[1] and occurs in approximately 10% of all neonates, but can incidence can be as high as 25% among very low birth weight (VLBW) babies (birth weight <1500 g), and approaches 50% among extremely low birth weight (ELBW) babies (birth weight <1000 g).[2] Thrombocytopenia is an important complication of sepsis and can occur in 25% of patients admitted in the NICU.[3] Early-onset thrombocytopenia develops in utero or within 72 hours of birth and has a varied etiology; late-onset thrombocytopenia is almost always caused by sepsis and necrotizing enterocolitis.[4] Virtually any organism capable of causing sepsis can induce thrombocytopenia. Riedler et al[5] found an 80%

incidence of thrombocytopenia in gram-negative septicemia and 65% incidence in gram-positive septicemia. In recent years fungal sepsis is increasingly associated with thrombocytopenia, especially in ELBW babies.6 A fall in the platelet count is an early marker of sepsis and in fact can be used as a screening procedure for early detection of sepsis, especially in NICU settings.

We designed a prospective study with the aim of learning the incidence of thrombocytopenia in blood-culture positive sepsis and to study the influence of parameters like birthweight, gestational age and sepsis-associated comorbidities like necrotizing enterocolitis (NEC) and disseminated intravascular coagulation (DIC) on platelet response. The duration of thrombocytopenia in relation to type of organism and mortality with respect to degree of thrombocytopenia were also studied.

## PATIENTS AND METHODS

The study was performed prospectively in the neonatology unit of the Sher-I-Kashmir Institute Of Medical Sciences (SKIMS), which is a tertiary care centre, from January 2002 to December 2006 with prior approval by hospital ethical committee. Two hundred infants were included. All the babies were born in-house. An eligibility criterion was the presence of documented nosocomial LOS, defined by the presence of clinical features of sepsis along with a positive blood culture. Coagulase-negative staphylococcal (CoNS) sepsis was defined in our study as clinical signs of sepsis with a minimum of two positive blood cultures. For all patients in the nursery, it is our policy to do a routine baseline workup, which includes complete blood count (CBC), C-reactive protein (CRP) estimation, bacterial blood culture and fungal culture. Babies with early onset thrombocytopenia were excluded from the study. Infants with symptoms of sepsis (respiratory distress, poor feeding, lethargy, shock, disseminated intravascular coagulation, temperature instability) had a repeat sepsis evaluation performed. The evaluation CoNSisted of a CBC, including platelet indices, blood culture (bacterial and fungal), and serial CRP estimation. In case of clinical bleeding coagulogram with d-dimer ELISA was performed. Blood samples were drawn from peripheral venous puncture or through a central catheter. CBCs were performed by using Coulter counter (Sismax ST 3000). Serial platelet counts were performed in thrombocytopenic patients until platelet counts were normalized. Relevant data like sex, gestational age, birth weight, antenatal history, history of pregnancy-induced hypertension (PIH), mode of delivery, Apgar score, and hospital stay were recorded in all the study patients. Thrombocytopenia

was defined as a platelet count of $< 150 \times 10^9/L$ and was classified by severity (mild if the platelet count was between 100 and $<150 \times 10^9/L$, moderate if the count was between 50 and $<100 \times 10^9/L$, and severe if the platelet count nadir was $<50 \times 10^9/L$). Mortality was defined as death before discharge. Infants who were discharged to home were categorized as survivors.

Data is expressed as mean and standard deviation, and percentages. The intergroup comparison was done by the t test and ANOVA (F-test).

## RESULTS

Two hundred cases of nosocomial sepsis were identified. Thrombocytopenia developed in 119 (59.5%) patients. Among the thrombocytopenic patients, 54 (27%) were diagnosed as mild, 40 (20%) as moderate, and 25 (12.5%) as severe.

We compared neonatal data between the patients without thrombocytopenia and with mild, moderate and severe thrombocytopenia (Table 1). The birth weight (grams) and gestational age (weeks) were significantly lower among patients with moderate (1816 [562] g, 33.73 [2.9] weeks) and severe thrombocytopenia (1484 [620] g, 31.84 [3.90] weeks) compared with patients with a normal platelet count (2473 [628] g, 36.95 [2.77] weeks) and mild thrombocytopenia (2387 [615] g, 36.09 [3.16] weeks) (P<.01). However, the difference in gestational age and birth weight between the patients with no thrombocytopenia and mild thrombocytopenia was insignificant (P>.05).

The incidence of NEC was more in patients with severe thrombocytopenia (6/25, 24%) and moderate thrombocytopenia (8/40, 20%) than in patients with mild thrombocytopenia (1/54, 5.90%), and no thrombocytopenia (2/81, 11.80%). (P<.01). Similarly DIC was more commonly found in patients with severe thrombocytopenia (22/25, 88.0%) and moderate thrombocytopenia (10/40, 25.0%) (P<.01). Mortality was significantly more in patients with DIC. The presence of DIC among survivors was 17/159 versus non-survivors 19/41, P<.01). No differences were observed in maternal age, parity, gender, mode of delivery, Apgar score, or TORCH positivity among the groups.

Of the 200 patients with documented nosocomial sepsis, 125 (62.5%) were infected with *Klebsiella pneumoniae* and 75 (60.0%) developed thrombocytopenia (Table 2). In 31 patients (15.5%) combined fungal (*Candida* in all patients) and bacterial sepsis (*Klebsiella* 20, CoNS 8, *Enterococcus* 2, *E coli* 1, *S aureus* 1) was documented. All the patients in this group were LBW (1170 [890]). The incidence of thrombocytopenia was significantly higher in this group (28/31), 90.3%)

# original research report

**Table 1.** Demographic and clinical characteristics of the study groups.

| Characteristics | No thrombocytopenia (n=81) | Thrombocytopenia (n=119, 59.5%) | | | P value |
|---|---|---|---|---|---|
| | | Mild (n=54, 27%) | Moderate (n=40, 20%) | Severe (n=25, 12.5%) | |
| Gestational age (weeks) | 36.95 (2.77) | 36.09 (3.16) | 33.73 (2.99) | 31.84 (3.90) | <.01 |
| Birth weight (grams) | 2473 (628) | 2387 (615) | 1816 (562) | 1484 (620) | <.01 |
| Gender | | | | | |
| Male | 39 (39%) | 23 (23%) | 27 (27%) | 11 (11%) | NS |
| Female | 42 (42%) | 31 (31%) | 13 (13%) | 14 (14%) | |
| Maternal age (years) | 30.5 (4) | 31.4 (3.8) | 30.4 (6) | 32.2 (4) | NS |
| Mode of delivery | | | | | |
| NVD | 55/81 (62.9%) | 38/54 (70.3%) | 25/40 (62.5%) | 12/25 (48%) | NS |
| LSCS | 26/81 (32%) | 16/54 (29.6%) | 15/40 (37.5%) | 10/25 (52%) | |
| Low birth weight | 39 (32.8%) | 25 (28.6%) | 34 (28.6%) | 21 (17.6%) | <.01 |
| TORCH +ve | - | - | 2 (66.7%) | 1 (33.3%) | NS |
| DIC | - | 4/54 (7.4%) | 10/40 (25%) | 22/25 (88%) | <.01 |
| NEC | 2/81 (2.46%) | 1/54 (1.85%) | 8/40 (20%) | 6/25 (24%) | <.01 |
| Mortality | 9/81 (11%) | 8/54 (14.8%) | 8/40 (20%) | 16/25 (64%) | <.01 |

Data are mean (standard deviation) or number (percentages). NVD: normal vaginal delivery, LSCS: lower segment cesarean section, TORCH: toxoplasmosis, rubella, cytomegalovirus, herpex simplex

**Table 2.** Organism-specific response to thrombocytopenia.

| Type of organism | No thrombocytopenia | Thrombocytopenia | | |
|---|---|---|---|---|
| | | Mild | Moderate | Severe |
| *Klebsiella* | 50/125 (40.0%) | 39/125 (31.2%) | 23/125 (18.4%) | 13/125 |
| Mixed fungal and bacterial sepsis | 3/31 (9.6%) | 9/31 (29.0%) | 9/31 (29.0%) | (10.4%) |
| CoNS | 14/21 (66.6%) | 3/21 (14.2%) | 3/21 (14.2%) | 10/31 (32.2%) |
| *S aureus* | 5/7 (71.4%) | 1/7 (14.2%) | 1/21 (14.2%) | 1/21 (4.76%) |
| *Candida* | 2/6 (33.3%) | --- | 3/6 (50.0%) | --- |
| *Enterococcus* | 3/5 (60.0%) | 1/5 (20.0%) | 1/5 (20.0%) | 1/6 (16.6%) |
| *E coli* | 4/5 (80.0%) | 1/5 (20.0%) | --- | --- |

(*P*<.01). The incidence of CoNS sepsis in our study population was 10.5% (21/200) and among these 7 (33.3%) patients had at least 1 episode of thrombocytopenia. Six patients (3%) in our series had a positive culture for *Candida albicans,* and the incidence of thrombocytopenia in those patients was 66.6% (4/6). All were ELBW babies. Other organisms isolated were *E coli* (5/200, 2.5%), *S aureus* (7/200, 3.5%) and *Enterococcus* (5/200, 2.5%).

The mean duration of thrombocytopenia was significantly higher in patients of combined bacterial and fungal sepsis (P<.05) as compared to isolated fungal sepsis and *Klebsiella sepsis*. The mean duration of thrombocytopenia was lowest in CoNS sepsis (Figure 1).

The overall mortality rate in our study population was 20.5% (41/200) (Figure 2). Mortality was significantly higher in patients with thrombocytopenia (32/119, 26.8%) than in the non-thrombocytopenic
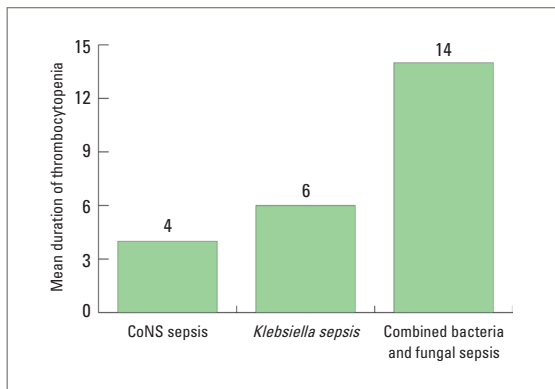
**Figure 1.** Mean duration of thrombocytopenia among different organisms.
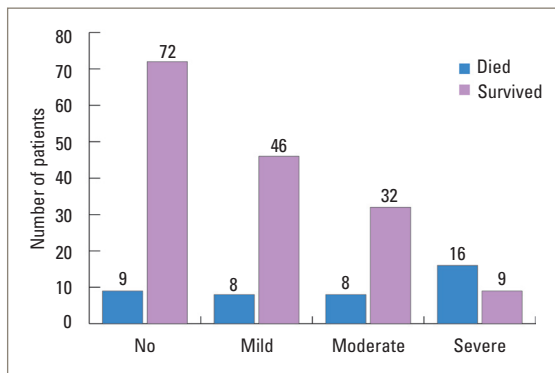


**Figure 2.** Mortality in different study groups.

group (9/81, 11.1%) (*P*<.01). Mortality was highest in patients with severe thrombocytopenia (16/25, 64.0%) as compared to patients with moderate thrombocytopenia (8/40, 20.0%) and mild thrombocytopenia (8/54, 14.8%).

## DISCUSSION

Platelets are among the least studied cells in sepsis, yet it has been well described for more than 30 years that patients with sepsis often have low platelet counts and that intravenous injection of lipopolysaccharides (LPS) into mice induces rapid thrombocytopenia.[7] Within a few minutes of the intravenous injection of lipopolysaccharide (LPS) into mice, platelets accumulate primarily in the liver and in lungs.[8] Although the mechanisms of activation and recruitment remain unclear, they are thought to be related to the cascade of endogenous mediators released rather than to a direct response to LPS.

We conducted this study to see the incidence of late-onset thrombocytopenia among inborn babies with documented nosocomial sepsis irrespective of ges-

tational age and birth weight. We observed that 59.5% of babies with documented sepsis developed a platelet count <150×10$^9$/L with 27% presenting with mild, 20% moderate and 12% with severe thrombocytopenia. LOS is an important risk factor for thrombocytopenia and can involve up to 80% of septic babies.[9] In a study by Naeme et al[10] on adult patients, thrombocytopenia occurred in about 75% of patients. Furthermore, we observed that the incidence of thrombocytopenia was more common in LBW babies (67.1%) as compared to normal birth weight babies (48.1%, *P*<.05). The former group developed a lower platelet nadir. This was similar to observation of many authors.[11,12]

Profound thrombocytopenia occurs in humans with sepsis and in mice administered LPS. Growing evidence indicates that, in addition to their hemostatic role, platelets play an important role in linking innate and adaptive immune responses.[13] Platelets express a family of signaling receptors, known as Toll-like receptors (TLRs) and they recognize a variety of molecular structures found on bacteria, viruses, and fungi.[14-16] Recognition of these "danger" molecules leads to TLR signaling, which in turn leads to the production of several proinflammatory cytokines.[17] It appears that TLRs are sentinels of the innate immune system and are essential for the eventual stimulation of adaptive immunity against invading microorganisms. TLR expression on platelets has a role to play during infectious inflammation and atherosclerotic vascular disease. This is perhaps best exemplified by the clinical observation of severe thrombocytopenia associated with sepsis.[18] Some septic babies also develop thrombocytopenia on the basis of DIC, but most have thrombocytopenia without laboratory evidence of DIC. In a study of septic adults by Naeme et al,10 DIC was present in a small minority, and these were a subgroup who had a platelet count of less than 50 000/L. Our data revealed that DIC was associated in almost 25% of patients and the majority of them belonged to severe thrombocytopenia group.

NEC is an important risk factor for late-onset thrombocytopenia. Ververidis et al[19] showed that thrombocytopenia was a common finding in NEC. We confirmed the same association. The incidence of NEC was significantly higher in patients with moderate (20%) and severe thrombocytopenia (24%). We demonstrated an association of fungal sepsis and gram negative (*Klebsiella*) sepsis with thrombocytopenia, an association that has been confirmed in many studies.[20] In fact, we observed that the incidence of thrombocytopenia was highest among the patients with combined fungal and bacterial sepsis (90.3%) (most of whom had *Klebsiella* isolated from blood), followed by isolated fungal sepsis (66.6%)

# original research report

and *Klebsiella sepsis* (60.0%). CoNS sepsis was less frequently associated with thrombocytopenia in our series (33.3%). Padovani et al[21] reported 26 VLBW neonates who had fungal sepsis and noted that 19 (73%) presented with thrombocytopenia. In fact, it is recommended to start empiric antifungal treatment when thrombocytopenia is seen in ELBW infants who are clinically septic with a nosocomial infection while awaiting culture reports.[22] Similarly, gram-negative sepsis is an important cause of thrombocytopenia in humans.

Thrombocytopenia is itself associated with increased mortality in neonates[23,24] as well as in adult septic patients.[25] We observed that morality was significantly more common in thrombocytopenic patients (26.8%) than patients with a normal platelet count (11.1%) (*P*<.01). Mortality was higher among patients with severe thrombocytopenia.

Our study indicates that a large proportion (59.5%) of infants with nosocomial sepsis develop thrombocytopenia and the severity of thrombocytopenia is directly related to gestational age and birth weight. We were able to confirm that mixed fungal and bacterial sepsis were more frequently associated with thrombocytopenia with a significantly higher mean duration of thrombocytopenia. We demonstrated a significantly higher mortality in thrombocytopenic group than in the non-thrombocytopenic group. A larger controlled study could be undertaken in resource poor settings where the burden of sepsis and low birth weight is high, to test whether profound and persistent thrombocytopenia can be used as a surrogate marker for fungal sepsis. The study also gives some justification for empiric antifungal therapy in situations where fungal cultures are unavailable or unaffordable.

## REFERENCES

**1.** Stoll BJ, Gordon T, Korones SB, Late-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. J Pediatr. 1996; 129:63-71.

**2.** Beck-Sague CM, Azimi P, Fonseca SN, et al. Bloodstream infections in neonatal intensive care unit patients: results of a multicenter study. Pediatr Infect Dis J.1994; 13 :1110 -1116.

**3.** Castle V, Andrew M, kelton J, Giron D, Johnston M, Carter C. Frequency and mechanism of neonatal thrombocytopenia. J pediatr 1986; 108 : 749-755.

**4.** Murray NA, Howarth LJ, McCloy MP et al. platelet transfusion in the management of severe thrombocytopenia in neonatal intensive care unit patients. Transfuse Med 2002; 12:35-41.

**5.** Riedler GF, Straub PW, Frick PG. thrombocytopenia in septicemia: a clinical study for the evaluation of its incidence and diagnostic value. Helv Med Acta 36:23, 1971.

**6.** Benjamin DK Jr, Ross K, McKinney RE Jr, Benjamin DK, Auten R, Fisher RG. When to suspect fungal infection in neonates: clinical comparison of Candida albicans and Candida parapsilosis fungemia with coagulase negative staphylococcal bacteremia. Pediatrics. 2000; 106: 712-718.

**7.** Vincent JL, Yagusni A, Pradier O. Platelet function in sepsis. Crit Care Med. 2002; 30: s313-s317.

**8.** Shibazaki M, Kawabata Y, YokochiT, Nishda A, Takada H, Endo Y. Complement-dependent accumulation and degradation of platelets in the lung and liver induced following injection of lipopolysaccharides. Infect. Immun 1999. 67: 5186_5191.

**9.** Mondanlon HD, Ortiz OB: thrombocytopenia in neonatal infection. Clin Pediatr 20: 402; 1981.

**10.** Neame PB, Kelton JG, Walker IR. Thrombocytopenia in septicemia: the role of disseminated intravascular coagulation. Blood.1993; 56: 88.

**11.** McPherson RJ, Juul S. patterns of thrombocytopenia in hospitalized neonates. J Perinatol 2005; 215: 166-172.

**12.** Christen RD, Henry E, Wiedmeier SE et al. thrombocytopenia among extremely low birth weight neonates: data from a multihospital healthcare system. Journal of Perinatol 2006;26:348-353.

**13.** Bennett DE, Tian J, Robert JJ, et al. Platelet mediated modulation of adaptive immunity: a communication link between innate and adaptive immune compartments. Immunity. 2003;19:9-19.

**14.** Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF. A family of human receptors structurally related to Drosophila Toll. Proc Natl Acad Sci U S A. 1998;95:588-593.

**15.** Diebold SS, Kaisho T, Hemmi H, Akira S, Reis E,Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 2004;303:1529-1531.

**16.** Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol. 2003;21:335-376.

**17.** Adachi O, Kawai T, Takeda K, et al. Targeted disruption of the MyD88 gene results in loss of IL-1ªnd IL-18 mediated function. Immunity. 1998;9: 143-150.

**18.** Warkentin TE, Aird WC, Rand JH. Platelet endothelial interactions: sepsis, HIT, and antiphospholipid syndrome. Hematology. 2003;8:497-519.

**19.** Ververidis M, Kiely EM, Spitz L, Drake DP, Eaton S, Pierro A. Theclinical significance of thrombocytopenia in neonates with necrotizingenterocolitis. J Pediatr Surg. 2001; 36:799-803.

**20.** Guida JD, Kunig AM, Leef KH, et al.: Platelet count andsepsis in very low birth weight neonates: Is there an organism-specific response? Pediatrics 2003, 111 (6 Pt 1):141-145.

**21.** Padovani EM, michielutte F, Dall Agnola A. sepsis caused by candida in the neonatal period. Pediatr Med chir. 1997; 19:83.

**22.** McDonnell M, Isaacs D: neonatal systemic Candidiasis. J Pediatr Child Health 1995; 31:490.

**23.** Mehta P, Vassa R, Neumann L, Karpatkin M. thrombocytopenia in the high- risk infant. J Pediatr. 1980; 97: 791-794.

**24.** Lea B Anna P, Shakuntala N, Rajeev M. thrombocytopenia related neonatal outcome in preterms. Indian journal of pediatrics 2007; 74:269-274.

**25.** Baughman RP, Lower EE, Flessa HC,Tollerud DJ. Thrombocytopenia in the intensive care unit. Chest 1993;104: 1243-1247.