Aswan University Medical Journal, volume 5 / No.3/ September 2025 (36-48) Online ISSN: **2735-3117**

ORIGINAL ARTICLE

Relationship between Severity of Neonatal Thrombocytopenia and Prophylactic Platelet Transfusion in NICU

Ashraf A. Meabed[1], Entesar Awn Allah Gaber Barsi[1*], Magda F Gabri[1], Khalid IA Elsayh[2], Hanan Mohammed abd el moneim[1].

[1]Pediatrics Department, Faculty of Medicine, Aswan University, Egypt

[2]Pediatrics Department, Faculty of Medicine, Assuit University, Egypt

ABSTRACT

Keyword: NT, NICU, Hemorrhage, NEC, Sepsis.

*** Corresponding author:**
Entesar Awn Allah Gaber Barsi

Mobile+201065035578

E-mail:
entesarawny2016@gmail.com

Background: Prophylactic platelet transfusion carries higher risk of complications, however, there no solid proof that a newborn's platelet count and severe bleeding are related. **Objectives:** The current study aimed to explore the association between NT severity and platelet transfusion and the possible complications based on NT severity. **Methodology:** 140 newborns hospitalized to the Neonatal Intensive Care Unit with a platelet count <100,000/ml participated in this randomized controlled experiment. **Results:** cases with severe NT had higher rates of sepsis and NEC (53% and 26%) than those with moderate NT (34% and 9.5%). Also, need for platelet transfusion was higher among severe (70%) vs moderate NT (27%) cases (p<0.001).In addition, NEC was higher in cases needed transfusions and proportion of patients with hemorrhage was higher in those needed transfusion or with hemorrhage. Likewise, the need for transfusion and cases with hemorrhage was associated with higher prevalence of mortality (33.3% and 83%) than no need (1.4% and 2.6%) (p<0.001). **Conclusion:** the current study shredded the light on the positive relationship between NT severity, need for transfusion and hemorrhage with the patients' main outcomes i.e., hemorrhage and mortality.

INTRODUCTION

Neonatal thrombocytopenia (NT) is a common hemostatic abnormality among newborn in the Neonatal Intensive Care Unit (NICU), which had positive correlation with prematurity **(1)**. Different etiologies for NT have been identified either immunologic or non-immunologic **(2)**. The following criteria were used to determine the degree of thrombocytopenia: A platelet count of 100,000 to 150,000/μL is considered mild; a platelet count of 50,000 to <100,000/μL is considered moderate; a platelet count of 30,000 to <50,000/μL is considered severe; and a platelet count of < 30,000/μL is considered very severe **(3)**.

Prophylactic platelet transfusion using 50,000/μL at threshold carries higher risk of complications i.e., death or major bleeding compared with 25,000/μL **(4**). Thus, the use of a restrictive neonatal platelet transfusion guideline is advocated to decrease potentially hazardous platelet transfusions in NICUs, while the usefulness of prophylactic platelet transfusions in neonates is disputed **(5)**. Using criteria based on the platelet mass index (PMI) may reduce platelet transfusion rates in the NICU, but more data from prospective studies is required **(6)**. Hence, severity of NT should be included to boost these guidelines.

The current study aimed to explore the association between NT severity and platelet transfusion and the possible complications based on NT severity.

PATIENTS AND METHODS

In the period from April 2022 to October 2023, 140 newborns with NT who were hospitalized to the NICU at Aswan University Hospitals participated in this single blinded Randomized Controlled Trial (RCT).

Using Stata v-16, a minimum sample of 124 neonates were required to reveal prophylactic platelet transfusion reduction of 20% (92% to 73%) with error probability of 5% and study power of 80% **(11)**. A 20% increase in the sample was applied to compensate for attrition and dropouts, hence the total sample became 140 (i.e., 70 per group).

Inclusion criteria were: NT cases who were admitted to the NICU and had a PLT count of <100,000/ml. However, those with hydrops fetalis, thrombocyte illnesses, coagulation problems, or significant congenital anomalies were excluded.

Randomization:

Automated random number generator was used for case distribution. According to eligibility, two equal groups with random assignment were created i.e., **Group-I**: consisted of 70 neonates who had platelet transfusions in accordance with the PLT count-based recommendation and **Group-II:** included 70 neonates with platelet transfusion according to PMI based guideline. Allocation was kept in sealed, opaque envelopes with sequential numbers. A Sysmex Xn 1000 Hematology Analyzer and a Mindray Hematology Analyzer BC-5150 were used to measure PLT counts and MPVs. Three milliliters of venous blood were anticoagulated using ethylene diamine tetra-acetic acid (EDTA). The following criteria were used to determine the degree of thrombocytopenia: A platelet count of 100,000 to 150,000/μL is considered mild; a platelet count of 50,000 to <100,000/μL is considered moderate; a platelet count of <50,000/μL is considered severe.

Procedure

Every case under study had routine investigation (complete blood count, or CBC), clinical examination, and detailed history taking. Clinical condition was categorized based on the amount of PLT transfusions received by subjects receiving one or more transfusions, as well as the total number of transfusions in both groups: I- ***Stable patients*** **(8)** either premature or full-term, II- ***Unstable patients*** and ***III- immediately pre or postoperative patients***. PLTs that were matched to the groups performed all of the transfusions. The PLTs were extracted from whole blood donations, which underwent leuco-filtration and were given in a dosage of 10–20 milliliters per kilogram of body weight. All transfusions were carried out using group-matched PLTs. Leuco-filtered whole blood donations were used to extract the PLTs, which were then administered at a dosage of 10–20 milliliters per kilogram of body weight.

Statistical analysis

IBM-SPSS version 26 was used to analyze the data **(9)**. Frequencies and percentages were used to display categorical data. In accordance with their distribution, numerical data were shown as mean and standard deviation (SD) or median and range after being examined for normality using the Shapiro-Walk test. Mann Whitney U test/Independent Sample T-test were used, as appropriate, to examine the difference in the mean/median between the two groups. Paired Sample T-test/Wilcoxon Sign test was used to explore the difference in mean/median within group before vs. after transfusion within each group. Chi-square test/Fisher's exact test was used to compare the difference in frequency between groups as convenient. A p-value of less than 0.05 was deemed significant.

Ethical considerations

The study's methodology was authorized by the Aswan University Faculty of Medicine's Institutional Ethics and Research Review Board (IRB). Before the trial was conducted, the neonates' caregivers signed a written informed consent form. The study adhered to STROBE standards for observational studies **(10)**, as well as Helsinki Declaration **(11)**, and was not abided with any incentives or rewards for participants or their guardians.

RESULTS

The recruited 140 neonates had gestational age of 26-40 weeks and weight of 800-4200 gm. Also, males represented about 57% of the studied cohort.

Table 1 showed the NT associated characteristics, it was found that 42% (n=59) of the sample had early-onset NT and about 58% (n=81) had late-onset NT. The median onset time was 4 (1-20) days. Also, nearly 53% had moderate NT and 47% have sever NT. Further, 47% (n=59) of the studied neonates needed platelets transfusion. Additionally, the median number of platelet transfusions per patient was 0 (0-7) and the total number of transfusions in all patients who needed transfusion was 110 Transfusions.

Table (1): Characteristics of thrombocytopenia among studied neonates

Variables	Total (n=140)	%
Onset of thrombocytopenia		
▪ Early onset	59	42.1%
▪ Late onset	81	57.9%
Onset day:		
▪ Mean ± SD	4.10 ± 2.93	
▪ Median (range)	4.0 (1.0 - 20.0)	
Severity of thrombocytopenia		
▪ Moderate (50,000-100,000)	74	52.9%
▪ Sever (Less than 50,000)	66	47.1%
Patients need platelets transfusion		

▪ Not need	74	52.9%
▪ Need	66	47.1%
Number of platelet transfusion for patient		
▪ Mean ± SD	0.79 ± 1.11	
▪ Median (range)	0.0 (0.0 - 7.0)	
Total number of transfusions in all patients need transfusion	**110 transfusions**	
Transfusion guidelines according to:		
▪ Platelet count guideline	70	50.0%
▪ Platelet mass index guideline	70	50.0%

Data expressed as Mean ± SD/median (range) or frequency (%)

As shown in figures 1 and 2, showed that 17% have post-transfusion hemorrhage namely, 11.5% pulmonary, 5.7% intracranial, 2.9% cutaneous, and 2.1% gastrointestinal. Regarding overall mortality, mortality rate was 16.4%.

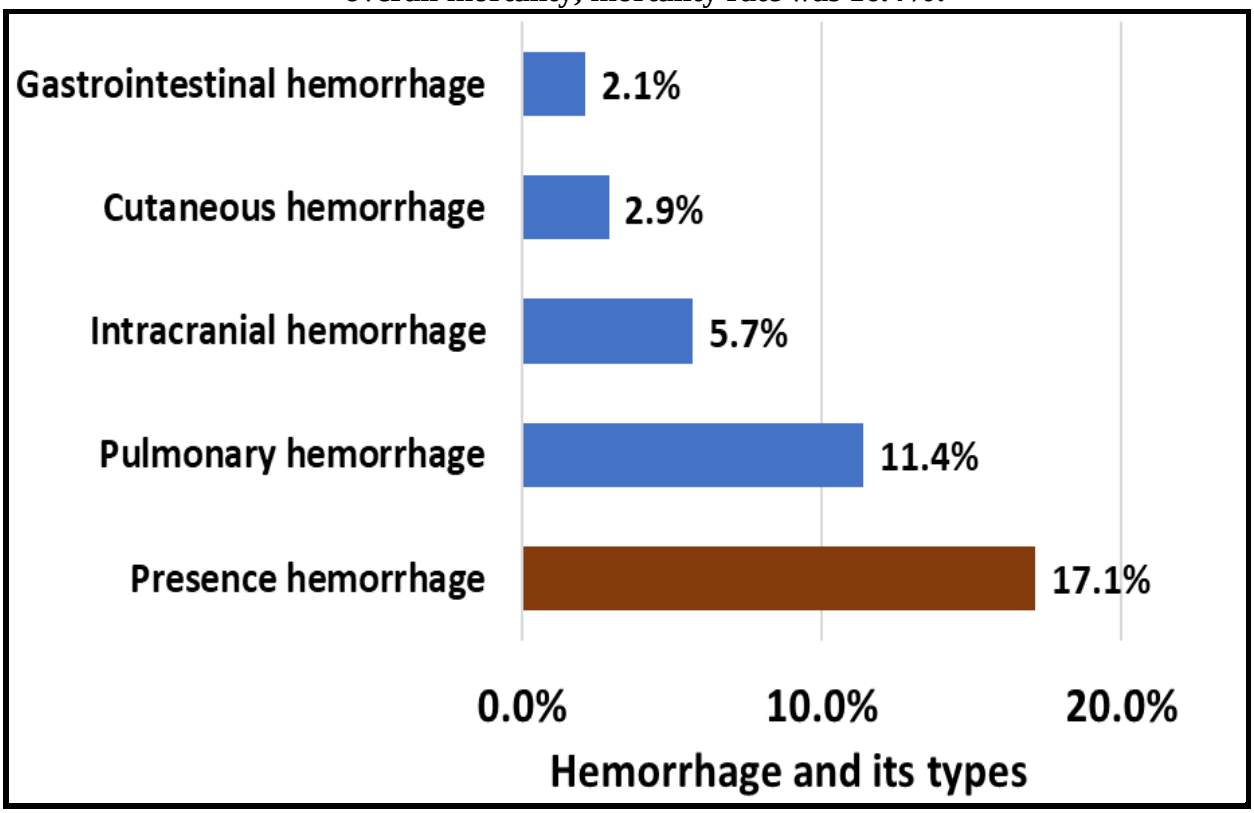

Figure (1): Hemorrhage among studied neonates with thrombocytopenia

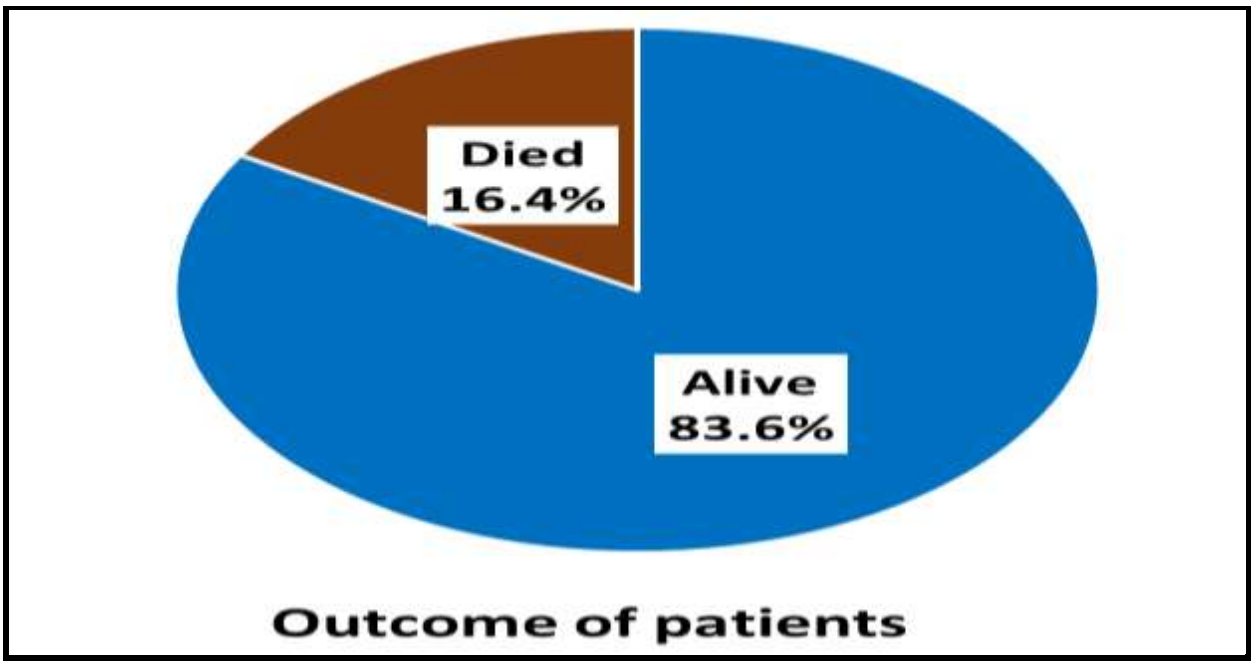

Figure (2): Outcome of studied neonates with thrombocytopenia

Table 2 illustrated relationship between patient characteristics and disease severity. For neonatal risk factors, cases with severe NT had significantly (p=0.022 and 0.011) higher rates of sepsis and NEC (53% and 26%) than those with moderate NT (34% and 9.5%). Also, need for blood transfusion was higher among severe (70%) vs moderate NT (27%) cases (p<0.001).

Table (2): Comparison of patient characteristics based on severity of thrombocytopenia

Variables	Severity of thrombocytopenia		P-Value*
	Moderate (n=74)	Sever (n=66)	
Gestational age (weeks)			
▪ Mean ± SD ▪ (range)	34.99 ± 3.1 (26 - 40)	34.88 ± 2.7 (30 - 40)	0.827
Weight (gm)			
▪ Mean ± SD ▪ (range)	2156.89 ± 770.8 (800 - 4000)	1954.7 ± 774.5 (1000 - 4200)	0.124
Gender			
▪ Males ▪ Females	41 (55.4%) 33 (44.6%)	39 (59.1%) 27 (40.9%)	0.660
Risk Factors			
Neonatal risk factors			
▪ Respiratory distress syndrome	40 (54.1%)	43 (65.2%)	0.182
▪ Prematurity	34 (45.9%)	39 (59.1%)	0.120
▪ Sepsis	25 (33.8%)	**35 (53.0%)**	**0.022**
▪ Hyperbilirubinemia	15 (20.3%)	14 (21.2%)	0.891
▪ Premature rupture of membrane	13 (17.6%)	15 (22.7%)	0.446
▪ Necrotizing enterocolitis	7 (9.5%)	**17 (25.8%)**	**0.011**
▪ Intrauterine growth restriction	12 (16.2%)	7 (10.6%)	0.333

Maternal risk factors			
▪ Pre-eclampsia	10 (13.5%)	17 (25.8%)	0.067
▪ ITP	2 (2.7%)	1 (1.5%)	0.999
▪ Blood incompatibility	6 (8.1%)	7 (10.6%)	0.611
▪ SLE	1 (1.4%)	2 (3.0%)	0.602
▪ Infant of diabetic mother	5 (6.8%)	4 (6.1%)	0.999
▪ Hepatitis B and C	3 (4.1%)	1 (1.5%)	0.622
▪ Corona virus disease	1 (1.4%)	2 (3.0%)	0.602
Patients need platelet transfusion			
▪ Not need	54 (73.0%)	20 (30.3%)	**<0.001**
▪ Need	20 (27.0%)	46 (69.7%)	
Hemorrhage	6 (8.1%)	18 (27.3%)	**0.003**
Type of hemorrhage			
▪ Pulmonary	2 (2.7%)	14 (21.2%)	**0.001**
▪ Intracranial	3 (4.1%)	5 (7.6%)	0.370
▪ Cutaneous	3 (4.1%)	1 (1.5%)	0.622
▪ Gastrointestinal	0 (0.0%)	3 (4.5%)	0.102
Outcome			
▪ Alive	69 (93.2%)	48 (72.7%)	**0.001**
▪ Dead	5 (6.8%)	18 (27.3%)	

Chi square test/Fisher Exact test compare proportion between groups
Independent Sample T test compare mean between two groups
Severity: Mild: 100,000-150,000. Moderate: 50,000- less than 100,000. Severe: less than 50,000.

there was no statistically significant difference between moderate and severe thrombocytopenia regarding gestational age, weight, gender, p value >0.05. Additionally, prevalence of hemorrhage (particularly pulmonary) was higher in severe vs moderate NT (21.2% vs 2.7%, p=0.001). Likely, mortality rate was higher in severe (27%) compared with moderate NT (6.8%) (p=0.001).

Comparison of patients' characteristics regarding the need for platelet transfusion was demonstrated in **table 3.** The mean weight was significantly lower among those needed transfusions vs those did not (1896.8 ± 739.1 vs 2208.5 ± 784.2, p=0.017). Further, NEC was higher in cases needed transfusions (24.2% vs 10.8%, p=0.035). Proportion of patients with hemorrhage was higher in those needed transfusion (particularly pulmonary [24%] and intracranial [12.4%]) in comparison with cases who did not need transfusion (pulmonary [0%] (p<0.001) and intracranial [0%] (p=0.002)). Likewise, the need for transfusion was associated with higher prevalence of mortality (33.3%) than no need (1.4) (p<0.001).

Table (3): Comparison of patient characteristics based on need for a platelet transfusion

Variables	Not need Platelet transfusion (n=74)	Need Platelet transfusion (n=66)	P-Value*
Gestational age (weeks)			
▪ Mean ± SD ▪ (range)	35.32 ±2.8 (26 - 40)	34.50 ± 2.9 (27 - 40)	0.092
Weight (gm)			
▪ Mean ± SD ▪ (range)	2208.51 ±784.2 (900 - 4200)	1896.8 ± 739.1 (800-3500)	**0.017**
Gender			
▪ Males ▪ Females	42 (56.8%) 32 (43.2%)	38 (57.6%) 28 (42.4%)	0.922
Risk Factors			
Neonatal risk factors			
▪ Respiratory distress syndrome	47 (63.5%)	36 (54.5%)	0.281
▪ Prematurity	33 (44.6%)	40 (60.6%)	0.058
▪ Sepsis	26 (35.1%)	34 (51.5%)	**0.051**
▪ Hyperbilirubinemia	20 (27.0%)	9 (13.6%)	**0.051**
▪ Premature rupture of membrane	14 (18.9%)	14 (21.2%)	0.735
▪ Necrotizing enterocolitis	8 (10.8%)	16 (24.2%)	**0.035**
▪ Intrauterine growth restriction	11 (14.9%)	8 (12.1%)	0.636
Maternal risk factors			
▪ Pre-eclampsia	15 (20.3%)	12 (18.2%)	0.755
▪ ITP	3 (4.1%)	0 (0.0%)	0.247
▪ Blood incompatibility	8 (10.8%)	5 (7.6%)	0.571
▪ SLE	2 (2.7%)	1 (1.5%)	0.999
▪ Infant of diabetic mother	6 (8.1%)	3 (4.5%)	0.500
▪ Hepatitis B, C	2 (2.7%)	2 (3.0%)	0.999
▪ Corona virus disease	2 (2.7%)	1 (1.5%)	0.999
Hemorrhage	3 (4.1%)	21 (31.8%)	**<0.001**
Type of hemorrhage			
▪ Pulmonary	0 (0.0%)	16 (24.2%)	**<0.001**
▪ Intracranial	0 (0.0%)	8 (12.1%)	**0.002**
▪ Cutaneous	4 (5.4%)	0 (0.0%)	0.122
▪ Gastrointestinal	0 (0.0%)	3 (4.5%)	0.102
Outcome			
▪ Alive ▪ Dead	73 (98.6%) 1 (1.4%)	44 (66.7%) 22 (33.3%)	**<0.001**

***Chi square test/Fisher Exact test compare proportion between groups**
Independent Sample T test compare mean between two groups

Table 4 showed the differences in patients' characteristics for occurrence of hemorrhage. There was significantly (p=0.019) lower mean weight among patients with hemorrhage than patients without hemorrhage (1724.6 ± 798.5 vs 2131.3 ± 756.6). For neonatal risk factors, there was a significantly (p=0.021) higher percentage of NEC in cases with hemorrhage vs those without (33.3% vs 13.8%). For maternal risk factors, there was a significantly (p=0.005) higher proportion of SLE among hemorrhagic cases vs non-hemorrhage (12.5% vs 0%). Regarding patient's outcome, there was significantly (p<0.001) higher mortality rate among patients with hemorrhage vs patients without hemorrhage (83% vs 2.6%).

Table (4): Comparison of patient characteristics based on occurrence of hemorrhage

Variables	No hemorrhage (n=116)	Hemorrhage (n=24)	P-Value*
Gestational age (weeks)			
▪ Mean ± SD ▪ (range)	35.12 ± 2.7 (26-40)	34.04 ± 3.7 (27-40)	0.190
Weight (gm)			
▪ Mean ± SD ▪ (range)	2131.29 ± 756.6 (900 - 4200)	1724.58 ± 798.5 (800 - 3500)	**0.019**
Gender			
▪ Males ▪ Females	69 (59.5%) 47 (40.5%)	11 (45.8%) 13 (54.2%)	0.219
Risk Factors			
Neonatal risk factors			
▪ Respiratory distress syndrome	66 (56.9%)	17 (70.8%)	0.206
▪ Prematurity	57 (49.1%)	16 (66.7%)	0.118
▪ Sepsis	47 (40.5%)	13 (54.2%)	0.219
▪ Hyperbilirubinemia	26 (22.4%)	3 (12.5%)	0.275
▪ Premature rupture of membrane	23 (19.8%)	5 (20.8%)	0.911
▪ Necrotizing enterocolitis	16 (13.8%)	8 (33.3%)	**0.021**
▪ Intrauterine growth restriction	17 (14.7%)	2 (8.3%)	0.410
Maternal risk factors			
▪ Pre-eclampsia	21 (18.1%)	6 (25.0%)	0.436
▪ ITP	2 (1.7%)	1 (4.2%)	0.434
▪ Blood incompatibility	13 (11.2%)	0 (0.0%)	0.125
▪ SLE	0 (0.0%)	3 (12.5%)	**0.005**
▪ Infant of diabetic mother	9 (7.8%)	0 (0.0%)	0.358
▪ Corona virus disease	3 (2.6%)	0 (0.0%)	0.999
Onset of thrombocytopenia			
▪ Early onset ▪ Late onset	49 (42.2%) 67 (57.8%)	10 (41.7%) 14 (58.3%)	0.959
Onset day			

▪ Mean ± SD ▪ Median (range)	4.05±2.90 4.00 (1.0-20.0)	4.12±3.15 4.00 (1.0-13.0)	0.784
Outcome			
▪ Alive ▪ Dead	113 (97.4%) 3 (2.6%)	4 (16.7%) 20 (83.3%)	**<0.001**

Data expressed as Mean ± SD or frequency (%). *Chi square test/Fisher Exact test compare proportion between groups, Independent Sample T test compare mean between two groups

Contrarily, there was no significant differences between groups regarding all other parameters.

DISCUSSION

Numerous studies have been conducted on platelet transfusion in neonates, and it is a common issue to identify strategies for reducing the incidence of platelet transfusion without raising the rates of bleeding or incorrect proof mortality **(12)**. Further testing is usually not necessary for individuals with mild NT, which is an accidental finding, if the platelet count stabilizes and improves on its own without treatment **(13)**. Similarly, babies delivered after a history or clinical indication of placental insufficiency and with moderate NT typically simply require platelet count monitoring to make sure it is temporary **(14)**. Bleeding is very common in neonates with severe NT. Neonates with very severe NT have higher incidence rate of cutaneous bleeding than those with severe NT **(15)**. Instant and short term adverse outcomes have been linked with severe NT at presentation **(16)**. In the 1st week of life, the majority of NT cases who were not transfused recovered spontaneously **(17)**. Babies with severe NT have the highest mortality rates, followed by those with mild to moderate disease and those without NT **(18)**.

This single blinded randomized controlled clinical trial was conducted on 140 neonates with NT at NICU, Pediatric department, Aswan University Hospital, Egypt. This study aimed to examine the association between NT severity disease outcomes and disease correlates. Recruited cases encompassed about 43% females and 57% males, with a mean weight of 2061.6 ± 766.4 grams and a mean gestational age of 34.9 ± 2.9 weeks.

In this study, NT had a median onset of 4 (1-20) days, with 42% experiencing early onset and 58% experiencing late onset. Similar to our results, **Zekry et al**. revealed that among newborns with NT, 84.5% had late onset **(19)**. Likely, in institution-based descriptive retrospective research conducted on 242 neonates with NT, a total of 205 (84.7%) of the neonates showed thrombocytopenia within the first 72 hours **(20)**. While, **Bolat et al**. found that out of 208 neonates with NT, 57% of the cases had early-onset **(21)**.

Numerous studies have found that infections such as late-onset sepsis, prematurity, prolonged hospitalization with intensive care interventions, the use of medications that suppress bone marrow or increase platelet destruction, and immune-mediated conditions are the main causes of the increased incidence of late-onset neonatal thrombocytopenia. Higher rates of thrombocytopenia seen in later phases of newborn care are caused by these variables, which are particularly common in critically sick neonates **(22)**.

As regard NT severity, about 53% have moderate and 47% have sever NT. This was disagreed with **Dahat et al** study who detected that the majority of neonates had moderate NT (37.4%); however, mild thrombocytopenia was seen in 32 (32%), and the rest of the neonates (31, 3%) had severe NT **(23)**. In a study by **Meena et al**., most of neonates had mild (46%), followed by moderate (35%)

and then severe NT (19%) **(24)**. The discrepancy between the current findings and other studies could be due to differences in study population characteristics, such as higher risk factors (e.g., maternal conditions like pre-eclampsia), variations in the definitions or thresholds for severity, timing of platelet count measurements, underlying causes of thrombocytopenia, or differences in sample size and study design.

Regarding platelets transfusion, about 53% did not need platelets transfusion, while 47% needed. The median number of transfusions was 0 (0-7). Total number of transfusions in all patients need transfusion was 110 Transfusions. Very small number of patients received PLT transfusions in **Kasap et al**. study as among the 395 thrombocytopenic patients, 30 (7%) received PLT transfusions. This lower rate in their NICU may be multifactorial; first, strict criteria are applied for PLT transfusions in their NICU to avoid unnecessary transfusions. In addition, our NICU includes both second level and third-level NICU patients, which may have resulted in the lower rates of severe thrombocytopenia and, consequently, PLT transfusions **(6)**. The difference in platelet transfusion rates in NT across studies can be attributed to variations in clinical guidelines, thresholds for transfusion, and institutional practices. Other factors include differences in the severity of thrombocytopenia in the study populations, underlying conditions (e.g., sepsis or NEC), and the availability of transfusion resources.

Based on NT severity, there was significantly higher percentage of sepsis and NEC among patients with severe thrombocytopenia. In line with our results, a retrospective cohort study of 422 NT cases detected that there was gradient association with Severity **(25)**. In Kasap et al. study, neonatal risk factors (sepsis, RDS, IUGR) showed positive correlation with severity) **(6)**. Likewise, it was reported that NT cases had higher proportion of need for platelets transfusion **(25)**. Consistent results for the mean number of platelet transfusions **(6-25).**

In addition, in our study there was statistically significantly higher percentage of hemorrhage particularly pulmonary among patients with severe NT, however, other types of hemorrhage were insignificant. Our result disagreed with **Von Lindern et al**. who did not find relationship between hemorrhage and NT severity. This suggests that bleeding in neonates depends on more variables than a platelet count alone **(25)**. In approximately one-third of cases, ICH was discovered before NT and this raises the question whether ICH can be explained as a cause or an effect **(26-27)**. Hemorrhage is probably due to pre-existing fragility in vessel wall structure and damaged blood vessels, amongst others by cytokines and/or a co-existing coagulopathy (**26**, **28**).

For patient's outcome, severe NT was related with higher mortality rate. In agreement, Al **Ghadeer et al**. detected that mortality rate was 5.3% in cases with mild compared to 21.8% in moderate NT and 25.7% in severe NT **(20)**. Also**, Kasap et al**. found similar results **(6)**.

Comparison of patient characteristics based on need for a platelet transfusion, there was significantly higher rate of NEC among those needing transfusions. Consistently**, Kasap et al**. detected that there was significant difference as regard sepsis and NEC between patients do not need transfusions and patients need transfusion. Likewise, they detected significant difference as regard ICH and mortality **(6)**. The current study reported similar results i.e., cases need transfusion had higher rates.

Moreover, there was significantly lower mean weight among cases with hemorrhage. In dis-concordance**, Kasap et al**. found that was no significant differences between patients with IVH and without IVH as regard gestational age, and birth weight **(6)**. As well, hemorrhagic cases had higher percentage of NEC. This could be explained by that NEC is closely related to hemorrhage in neonates with NT, as both conditions can exacerbate each other **(29)**. For maternal risk factors,

those with hemorrhage had higher proportion of mothers with SLE. Likely, **Zekry et al**. found that SLE and PIH were associated with bleeding NT **(19)**. Respecting outcome, there was significantly higher mortality rate among cases with hemorrhage. Similar results were reported by **Resch et al**., who detected that a mortality rate of 10.8% was significantly associated with bleeding signs **(30)**.

CONCLUSION

In conclusion, the current study shredded the light on the positive relationship between NT severity and patients' main outcomes i.e., hemorrhage and mortality. Also, the effect of neonatal risk factors on the need for transfusion, as well as the effect of such need on the patients' outcomes (hemorrhage and mortality). Likewise, the predictors of hemorrhage in NT cases were NEC among cases and SLE of their mothers, and higher mortality among those with hemorrhage.

Study Limitations

The current study had certain limitations. First, the study was conducted at a single center that jeopardize the external validity of the study. Second, in order to match groups, we did not assess potential confounding variables (i.e., the presence of alloimmunization or platelet refractoriness).

Conflict of interest: All authors declare that they have no conflicts of interest.

Financial Disclosures: None

Acknowledgement:

The authors would like to express their gratitude to the pediatric departments' administrative and medical staff at Aswan University Hospital for their continuous assistance and support. Without the assistance, encouragement, and consent of the caregivers of the patients who were recruited, this work would not have been possible.

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