Clinical Study

Neonatal Thrombocytopenia after Perinatal Asphyxia Treated with Hypothermia: A Retrospective Case Control Study

N. Boutaybi, F. Razenberg, V. E. H. J. Smits-Wintjens, E. W. van Zwet, M. Rijken, S. J. Steggerda, and E. Lopriore

1 Division of Neonatology, Department of Pediatrics, Leiden University Medical Center, 2333 ZA Leiden, The Netherlands
2 Department of Medical Statistics, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands

Correspondence should be addressed to E. Lopriore; e.lopriore@lumc.nl

Received 15 May 2014; Accepted 18 July 2014; Published 21 August 2014

Academic Editor: Naveed Hussain

Copyright © 2014 N. Boutaybi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Our objective was to estimate the effect of therapeutic hypothermia on platelet count in neonates after perinatal asphyxia. We performed a retrospective case control study of all (near-) term neonates with perinatal asphyxia admitted between 2004 and 2012 to our neonatal intensive care unit. All neonates treated with therapeutic hypothermia were included in this study (hypothermia group) and compared with a historic control group of neonates with perinatal asphyxia treated before introduction of therapeutic hypothermia (2008). Primary outcome was thrombocytopenia during the first week after birth. Thrombocytopenia was found significantly more often in the hypothermia group than in the control group, 80% (43/54) versus 59% (27/46) (P = .02). The lowest mean platelet count in the hypothermia group and control group was 97×10^9/L and 125×10^9/L (P = .06), respectively, and was reached at a mean age of 4.1 days in the hypothermia group and 2.9 days in the control group (P < .001). The incidence of moderate/severe cerebral hemorrhage was 6% (3/47) in the hypothermia group versus 9% (3/35) in the control group (P = .64).

In conclusion, neonates with perinatal asphyxia treated with therapeutic hypothermia are at increased risk of thrombocytopenia, without increased risk of cerebral hemorrhage.

1. Introduction

Thrombocytopenia, defined as a platelet count below 150×10^9/L, occurs in 1 to 5% of healthy term neonates [1, 2]. The prevalence of thrombocytopenia is reported to be much higher in sick neonates, ranging from 22 to 35% in those admitted to neonatal intensive care units [1–4]. Thrombocytopenia is associated with an increased risk of pulmonary, gastrointestinal, and intraventricular hemorrhage (IVH) [5].

One of the most common causes of early-onset thrombocytopenia (<72h of birth) in term neonates is perinatal asphyxia [2]. Perinatal asphyxia remains an important cause of morbidity and mortality of the full-term newborn and is still a major cause of death worldwide [6–10]. Therapeutic hypothermia, nowadays considered as the gold standard of treatment for perinatal asphyxia, reduces the risk of permanent brain injury and is associated with decreased rates of mortality and neurodevelopmental disability [6, 9, 11–13].

The protective effects of hypothermia are attributed primarily to a reduction of apoptosis and of inflammation [12].

In a recent Cochrane meta-analysis, therapeutic hypothermia was reported to increase the relative risk (RR) of thrombocytopenia in neonates with perinatal asphyxia (relative risk (RR): 1.21 (95% confidence interval (CI) 1.05–1.40)) compared to a control group [14]. However, research on the severity, course, and consequences of thrombocytopenia after therapeutic hypothermia is limited.

The aim of this study was to estimate the incidence, timing, and severity of thrombocytopenia during the first week after birth in neonates with perinatal asphyxia and to study the effect of therapeutic hypothermia on platelet count.

2. Methods

All (near-) term neonates (≥36 weeks’ gestation) with perinatal asphyxia admitted to the tertiary neonatal intensive care
hypothermia at a degree of 33.5°C in September 2008. Neonates were cooled by whole-body hypothermia as standard management for perinatal asphyxia. In our neonatal intensive care unit, platelet transfusions are given when: (1) platelet count is below 300×10^9/L, unstable infant, previous major bleeding or before planned surgery, or (3) platelet count is below 100×10^9/L in neonates with active bleeding [4].

2.3. Mortality, Neurologic Morbidity, and Other Clinical Data. The following postnatal data were recorded: mortality within 1 month after birth, neonatal seizures, treatment with anticonvulsants, neonatal sepsis (defined as a positive blood culture in a neonate with clinical signs of infection), small for gestational age (SGA, defined as a birth weight <10th centile), days on mechanical ventilation, number of hospital days in our neonatal intensive care unit, and hemorrhage on cranial ultrasound and/or magnetic resonance imaging (MRI). Cranial ultrasound scan (CUS) results were assessed for IVH and parenchymal hemorrhage. IVH was classified according to Papile et al. [15]. MRI scans were reviewed for presence of subdural, intraparenchymal, and/or intraventricular hemorrhage.

We classified mild hemorrhage as mild IVH (grade 1), punctate parenchymal hemorrhage, and minor subdural bleeds without parenchymal compression and/or shift detected by CUS and/or MRI. Moderate/severe IVH (≥grade 2), larger parenchymal hemorrhage, and large subdural hemorrhage causing parenchymal compression and/or shift were classified as moderate/severe hemorrhage.

2.4. Statistical Analysis. We calculated that group sizes of at least 45 infants were required to demonstrate a 25% difference in incidence of thrombocytopenia (75% versus 50%) with a significance of 0.05 and a power of 80%, by one-tailed analysis. Chi-square tests or Fisher’s exact tests were applied to analyze categorical variables, as appropriate. For comparison of continuous variables, independent-sample t-test was used. Odds ratios (OR) and 95% CI were calculated by univariate logistic regression. All reported P values were
Table 1: Baseline characteristics of the study population.

|                        | Hypothermia group | Control group | P value |
|------------------------|-------------------|---------------|---------|
| Birth weight—grams    | 3446 (625)        | 3352 (606)    | .45     |
| Gestational age—weeks  | 39.4 (1.55)       | 39.8 (1.85)   | .28     |
| Male gender—%          | 27 (50%)          | 18 (39%)      | .28     |
| Caesarean delivery—%   | 27 (50%)          | 28 (61%)      | .28     |
| Vacuum extraction—%    | 16 (30%)          | 15 (33%)      | .75     |
| Forceps delivery—%     | 2 (4%)            | 3 (7%)        | .66     |
| Shoulder dystocia—%    | 4 (7%)            | 1 (2%)        | .37     |
| Apgar at 5 min ≤ 5—%   | 51 (96%)          | 31 (67%)      | .00     |
| Arterial cord blood/blood gas pH < 7.0—% | 39 (72%) | 34 (74%) | .85 |
| Base excess ≤ −16 mmol/L—% | 33 (61%) | 24 (52%) | .37 |
| Lactate > 10 mmol/L—%  | 33 (61%)          | 22 (48%)      | .18     |

*Value given as mean (SD).

Figure 1: Flowchart showing the derivation of our population and the severity of thrombocytopenia.

Two-sided and were considered statistically significant at P < .05. Data analyses were performed using SPSS Statistics software (version 20.0, SPSS Inc., Chicago, IL, USA).

3. Results

During the study period, 118 (near-) term neonates (≥36 weeks’ gestation) with perinatal asphyxia were admitted to our neonatal intensive care unit. Neonates who died within 48 hours after birth (n = 15) and neonates with major congenital disorders (n = 3) were excluded. A total of 100 neonates met our inclusion criteria. In the hypothermia group 54 neonates were included and 46 neonates born in the period before introduction of therapeutic hypothermia, were included in the control group. The criteria for perinatal asphyxia were the same in the two groups. Baseline characteristics were similar between the hypothermia group and the control group, except for lower Apgar scores in neonates in the hypothermia group (see Table 1). The flow chart showing the derivation of our population and the severity of thrombocytopenia is shown in Figure 1.

Platelet counts were measured in 74/100 (74%) neonates at day 1 (day of birth) and at least once in the first week after birth in all neonates (100%). At day 1, the mean platelet count in the hypothermia group was 154 × 10⁹/L compared to 156 × 10⁹/L in the control group (P = .90). The lowest platelet count during the first week after birth was in the hypothermia group 97 × 10⁹/L compared to 125 × 10⁹/L in the control group (P = .06). The incidence of thrombocytopenia (< 150 × 10⁹/L) was significantly higher in the hypothermia group than in the control group, 80% (43/54) versus 59% (27/46), respectively (OR 2.75, 95% CI 1.14–6.66, P = .02). The subdivision of thrombocytopenia in mild, moderate, severe, and very severe was similar between the hypothermia group and the control group.
Table 2: Hematologic results of the study population.

|                        | Hypothermia group n = 54 | Control group n = 46 | P value | OR [95% CI] |
|------------------------|---------------------------|----------------------|---------|-------------|
| **Platelet count/transfusions** |                           |                      |         |             |
| Platelet count <150 × 10^9/L* | 43 (80%)                  | 27 (59%)             | .02     | 2.75 [1.14–6.66] |
| Platelet count at birth—×10^9/L* | 154 (77)                 | 157 (81)             | .90     | 1.00 [0.99–1.01] |
| Lowest platelet count in the first week after birth* | 97 (62)                   | 125 (78)             | .06     | 0.99 [0.99–1.00] |
| Day lowest platelet count* | 4.1 (1.8)                 | 2.9 (1.4)            | <.001   | 1.58 [1.21–2.06] |
| Neonates requiring platelet transfusions—n (%) | 15 (28%)                  | 10 (22%)             | .49     | 1.39 [0.55–3.47] |
| Number of platelet transfusions per neonate** | 0.00 (1)                  | 0.00 (0)             | .94     | 0.99 [0.74–1.32] |
| **Coagulation disorders/FFP transfusions** |                           |                      |         |             |
| PT—seconds* | 25.3 (15.0)              | 21.2 (16.7)           | .27     | 1.02 [0.98–1.06] |
| INR*       | 1.8 (1.1)                | 1.8 (1.5)            | .96     | 0.99 [0.69–1.43] |
| APTT—seconds* | 50.0 (20.1)            | 42.6 (23.8)           | .16     | 1.02 [0.99–1.04] |
| Fibrinogen—gram/L* | 1.6 (0.8)               | 2.0 (0.9)            | .08     | 0.60 [0.34–1.07] |
| Neonates requiring FFP transfusions—n (%) | 13 (24%)                  | 8 (17%)              | .41     | 1.51 [0.56–4.03] |
| Number of FFP transfusions per neonate** | 0.00 (0)                  | 0.00 (0)             | .81     | 0.95 [0.62–1.45] |

PT: prothrombin time; INR: international normalized ratio; APTT: activated partial thromboplastin time; FFP: fresh frozen plasma.

*Value given as median (IQR).

group: 24% (13/54) versus 17% (8/46) (P = .41), 24% (13/54) versus 13% (6/46) (P = .16), 15% (8/54) versus 15% (7/46) (P = .96), and 17% (9/54) versus 13% (6/46) (P = .61), respectively. The lowest platelet count in the hypothermia group was reached later compared to in the control group: at a mean age of 4.1 days (at day 5) versus 2.9 days (at day 3), after birth (P < .001) (see Figure 2). Coagulation data were collected from both groups. No significant differences in mean values of PT, APTT, INR, and fibrinogen were found between both groups. Further details on hematologic outcome in the study population are shown in Table 2.

3.1. Mortality, Neurologic Morbidity, and Other Clinical Data.

An overview of neurologic morbidity, clinical findings, and presence of hemorrhage on neuroimaging in both groups is presented in Table 3. Mortality rate in the hypothermia group was 31% (17/54) compared to 20% (9/46) in the control group (OR 1.89, 95% CI 0.75–4.78, P = .18). In the hypothermia group 83% (45/54) of the neonates were treated with anticonvulsants, compared to 74% (34/46) in the control group, with phenobarbital being the most commonly administered drug (alone or in combination with other drugs). The number of neonates requiring more than 2 types of anticonvulsants, number of hospital days, and number of ventilation days were similar in both groups. CUS was performed in all neonates. The incidence of IVH grades II–III was similar in both groups, 2% (1/54) in the hypothermia group and 2% (1/46) in the control group (OR 0.87, 95% CI 0.05–14.24, P = .92). On CUS no subdural and intraparenchymal bleedings were detected. MRI was performed in 82% (82/100) of neonates (hypothermia group 47/54; control group 35/46). The overall incidence of intracranial hemorrhage detected on MRI was similar in both groups, 23% (11/47) in the hypothermia group versus 23% (8/35) in the control group (OR 1.03, 95% CI 0.37–2.91, P = .95) and hemorrhages were mostly mild (Table 3). The incidence of moderate/severe hemorrhage was similar in both groups, 6% (3/47) versus 9% (3/35) (P = .64), respectively.
### Table 3: Morbidity, clinical findings, and presence of hemorrhage on neuroimaging.

| Hypothermia group n = 54 | Control group n = 46 | P value | OR [95% CI] |
|--------------------------|----------------------|---------|-------------|
| Seizures—𝑟 (%) | 45 (83%) | 34 (74%) | .25 | 1.77 [0.67–4.67] |
| Treatment with >2 types of anticonvulsants—𝑟 (%) | 18 (33%) | 8 (18%) | .07 | 2.38 [0.92–6.14] |
| Neonatal sepsis | 3 (6%) | 2 (4%) | .78 | 1.29 [0.20–8.10] |
| SGA | 0 (0%) | 2 (4%) | .12 | ∞ |
| IVH grades II-III on cranial US—𝑟 (%) | 1 (2%) | 1 (2%) | .92 | 0.87 [0.05–14.24] |
| Cerebral bleeding on MRI—𝑟 (%) | 11 (23%) | 8 (23%) | .95 | 1.03 [0.37–2.91] |
| Mild | 8 (17%) | 5 (14%) | .64 |  |
| Moderate/severe | 3 (6%) | 3 (9%) | .64 |  |
| Type of moderate/severe hemorrhage | | | | |
| Subdural | 0 (0%) | 0 (0%) | — |  |
| Intraparenchymal | 2 (4%) | 2 (6%) | .74 |  |
| Intraventricular | 2 (4%) | 1 (3%) | .76 |  |
| Mortality—𝑟 (%) | 17 (31%) | 9 (20%) | .18 | 1.89 [0.75–4.78] |
| Mechanical ventilation days* | 4.8 (2.94) | 3.6 (3.40) | .07 | 1.13 [0.99–1.28] |
| Hospital days* | 10.7 (7.07) | 10.4 (8.46) | .89 | 1.00 [0.95–1.06] |

SGA: small for gestational age; IVH: intraventricular haemorrhage; US: ultrasound; MRI: magnetic resonance imaging; ∞: infinite (because of zero value in one group); *value given as mean (SD).

### 4. Discussion

This study shows that the vast majority (80%) of neonates with perinatal asphyxia treated with hypothermia develop thrombocytopenia. This is an important topic especially since there is a movement to cool babies of lower gestational age without adequate safety data. The incidence of thrombocytopenia was almost threefold higher (OR 2.75) than in the control group with perinatal asphyxia treated without therapeutic hypothermia. The higher incidence of thrombocytopenia in the hypothermic group appears to be primarily an increase in the numbers of infants with mild or moderate thrombocytopenia.

Despite the increased rate of thrombocytopenia in the hypothermia group, we found no increased risk of intracranial hemorrhage. This is in accordance with previous studies showing no association between thrombocytopenia and the occurrence of major intracranial hemorrhage [4, 5, 16].

The incidence of thrombocytopenia in neonates treated with therapeutic hypothermia reported in the literature varies greatly from 3% to 65% [6–10, 17]. Differences in reported incidence are probably related to methodological differences between the studies, including different definitions for perinatal asphyxia, differences in timing of platelet count measurement, and variations in number of included patients. We found a difference in the course of platelet count between the hypothermia group and control group (see Figure 2). The lowest platelet count in the hypothermia group was reached at a later stage compared to the control group (day 5 versus day 3). This difference suggests an additional effect of hypothermia on platelet count in infants with perinatal asphyxia.

Our findings are in agreement with recent studies reported in the literature. In a recent Cochrane review (2013), eight randomized studies comparing platelet count in the hypothermia group with a control group were identified [14]. Meta-analysis of these eight trials showed a relative risk of thrombocytopenia in the hypothermia group of 1.21 (95% CI 1.05–1.40) compared to the control group [14]. This was in accordance with another systematic review from Shah et al. in 2007 (RR 1.51, 95% CI 1.09–2.10) [12]. In this review thrombocytopenia was defined as a platelet count below $100 \times 10^9/L$ and three randomized studies were analyzed [7, 8, 17]. Up to now, no individual randomized study found a difference in thrombocytopenia in the hypothermia group as compared to the control group [6–10, 17]. This correlation was only found in reviews after meta-analysis of different randomized studies [12, 14].

According to the recent Cochrane review, the mode of hypothermia (head cooling with mild systemic versus whole-body) may influence the incidence of thrombocytopenia. A slight increase (statistical) in risk of thrombocytopenia was detected in the group of infants treated with selective head cooling [14].

Hypothermia is known to decrease platelet function and platelet number [18]. The production of thromboxane B2, which has a role in clot formation, is dependent on temperature: lowering of the body temperature causes a reversible platelet dysfunction [18]. Hypothermia is also known to increase PT and APTT [19]. Enzymatic reactions of the coagulation cascade are inhibited by hypothermia [19]. However, in our study we found no differences in PT, APTT, and number of neonates requiring FFP transfusions. The occurrence of coagulation disorders was similar in both groups.

Because of the retrospective design the results of this study should be interpreted with care. Despite the fact that both groups in our study had the same criteria for perinatal asphyxia, the hypothermia group had lower Apgar scores. The higher incidence of thrombocytopenia in the hypothermia
group could partly be due to the more severe degree of perinatal asphyxia. However, arterial cord blood values and other clinical parameters were similar in both groups suggesting that differences between groups were probably minimal. A further limitation of our study is the small number ($n = 100$) of included neonates. Larger prospective studies should be conducted to determine the exact mechanism that contributes to the occurrence of thrombocytopenia by hypothermia. Randomized controlled trials are not ethical anymore because of the proven protective effect of therapeutic hypothermia.

In conclusion, this is the first study focusing on the incidence, severity, and course of thrombocytopenia after treatment with hypothermia. We conclude that therapeutic hypothermia increases the risk of thrombocytopenia in neonates after perinatal asphyxia, without increased risk of cerebral hemorrhage. Thrombocytopenia lasts longer and the nadir of platelet count is reached a couple of days later in neonates treated with hypothermia.

**Abbreviations**

- APTT: Activated partial thromboplastin time
- CI: Confidence intervals
-CUS: Cranial ultrasound scan
-FFP: Fresh frozen plasma
-INR: International normalized ratio
-IVH: Intraventricular hemorrhage
-MRI: Magnetic resonance imaging
-OR: Odds ratios
-PT: Prothrombin time
-RR: Relative risk
-SGA: Small for gestational age.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

**References**

[1] S. Chakravorty, N. Murray, and I. Roberts, “Neonatal thrombocytopenia,” *Early Human Development*, vol. 81, no. 1, pp. 35–41, 2005.

[2] I. A. G. Roberts and N. A. Murray, “Thrombocytopenia in the newborn,” *Current Opinion in Pediatrics*, vol. 15, no. 1, pp. 17–23, 2003.

[3] M. C. Sola, A. Del Vecchio, and L. M. rimsza, “Evaluation and treatment of thrombocytopenia in the neonatal intensive care unit,” *Clinics in Perinatology*, vol. 27, no. 3, pp. 655–679, 2000.

[4] J. S. von Lindern, T. van den Bruele, E. Lopriore, and F. J. Walther, “Thrombocytopenia in neonates and the risk of intraventricular hemorrhage: a retrospective cohort study,” *BMC Pediatrics*, vol. 11, article 16, 2011.

[5] V. L. Baer, D. K. Lambert, E. Henry, and R. D. Christensen, “Severe thrombocytopenia in the NICU,” *Pediatrics*, vol. 124, no. 6, pp. e1095–e1100, 2009.

[6] D. V. Azzopardi, B. Strohm, A. D. Edwards et al., “Moderate hypothermia to treat perinatal asphyxial encephalopathy,” *The New England Journal of Medicine*, vol. 361, no. 14, pp. 1349–1358, 2009.

[7] P. D. Gluckman, J. S. Wyatt, D. Azzopardi et al., “Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial,” *The Lancet*, vol. 365, no. 9460, pp. 663–670, 2005.

[8] A. J. Gunn, P. D. Gluckman, and T. R. Gunn, “Selective head cooling in newborn infants after perinatal asphyxia: a safety study,” *Pediatrics*, vol. 102, no. 4, pp. 885–892, 1998.

[9] S. E. Jacobs, C. J. Morley, T. E. Inder et al., “Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial,” *Archives of Pediatrics and Adolescent Medicine*, vol. 165, no. 8, pp. 692–700, 2011.

[10] S. Shankaran, A. R. Laptook, R. A. Ehrenkranz et al., “Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy,” *New England Journal of Medicine*, vol. 353, no. 15, pp. 1574–1584, 2005.

[11] D. J. Eicher, C. L. Wagner, L. P. Katikaneni et al., “Moderate hypothermia in neonatal encephalopathy: efficacy outcomes,” *Pediatric Neurology*, vol. 32, no. 1, pp. 11–17, 2005.

[12] P. S. Shah, A. Ohlsson, and M. Perlman, “Hypothermia to treat neonatal hypoxic ischemic encephalopathy: systematic review,” *Archives of Pediatrics and Adolescent Medicine*, vol. 161, no. 10, pp. 951–958, 2007.

[13] G. Simbruner, R. A. Mittal, F. Rohlmann et al., “Systemic Hypothermia after Neonatal Encephalopathy: outcomes of neo.nEURO.networkRCT,” *Pediatrics*, vol. 126, no. 4, pp. e771–e778, 2010.

[14] S. E. Jacobs, M. Berg, R. Hunt, W. O. Tarnow-Mordi, T. E. Inder, and P. G. Davis, “Cooling for newborns with hypoxic ischaemic encephalopathy,” *The Cochrane Database of Systematic Reviews*, vol. 1, Article ID CD003311, 2013.

[15] L. A. Papile, J. Burstein, R. Burstein, and H. Koffler, “Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm,” *Journal of Pediatrics*, vol. 92, no. 4, pp. 529–534, 1978.

[16] S. J. Stanworth, “Thrombocytopenia, bleeding, and use of platelet transfusions in sick neonates,” *The American Society of Hematology. Education Program*, vol. 32, no. 1, pp. 18–24, 2012.

[17] D. J. Eicher, C. L. Wagner, L. P. Katikaneni et al., “Moderate hypothermia in neonatal encephalopathy: safety outcomes,” *Pediatric Neurology*, vol. 32, no. 1, pp. 18–24, 2005.

[18] C. R. Valeri, G. Cassidy, and S. Khuri, “Hypothermia-induced reversible platelet dysfunction,” *Annals of Surgery*, vol. 205, no. 2, pp. 175–181, 1987.

[19] M. J. Rohrer and A. M. Natale, “Effect of hypothermia on the coagulation cascade,” *Critical Care Medicine*, vol. 20, no. 10, pp. 1402–1405, 1992.