Study of Platelet Count and Mean Platelet Volume According to the Use or non-use of Therapeutic Hypothermia

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ABSTRACT

Background: We examined changes in mean platelet volume (MPV) and platelet count according to the use or nonuse of therapeutic hypothermia for neonatal hypoxic ischemic encephalopathy (HIE).

Methods: We divided the newborn infants who were diagnosed as having HIE into the therapeutic hypothermia group (TH group, 24 infants) and non-therapeutic hypothermia group (non-TH group, 31 infants) and examined perinatal information and platelet count and MPV on admission, postnatal day 5, and postnatal day 7.

Result: In the TH group, an increase in MPV was found from admission to postnatal day 5 (p=0.03), whereas no change was found from postnatal day 5 to day 7 (p=0.36). In the non-TH group, MPV increased from admission to postnatal day 5 (p<0.01) and did not change from postnatal day 5 to day 7 (p=0.71). Within the two groups, a high level of MPV was found only on postnatal day 7 in the TH group (p<0.01). A negative correlation was found between platelet count and MPV on admission and on postnatal days 5 and 7 in the TH group (admission: p=0.02, R=0.46; postnatal day 5: p=0.03, R=0.43; postnatal day 7: p=0.03, R=0.42), but no correlation was found at any time point in the non-TH group (admission: p=0.93, R=0.11; postnatal day 5: p=0.07, R=0.28; postnatal day 7: p=0.58, R=0.13).

Conclusion: Even if therapeutic hypothermia was implemented early after birth, we presumed that the bone marrow maintained the platelet count by increasing platelet production as the platelet count decreased.

Keywords: Mean Platelet Volume, Platelet Count, Therapeutic Hypothermia, Neonatal Hypoxic Ischemic Encephalopathy

Introduction

Therapeutic hypothermia is a treatment to lower the body temperature to 34°C soon after the birth of newborn infants who are born with neonatal asphyxia and are in a poor perinatal state. The treatment requires attention because factors accompanying a state of hypothermia together with underlying disease are related to bleeding tendency. An association between hypothermic circulatory arrest and bleeding tendency has been reported in adults.1–6 Other than deficiencies in coagulation factors, the causes include a wide variety of factors such as platelet activation and aggregation, temporary incorporation into the reticuloendothelial system (sequestration), and adsorption in the artificial cardiopulmonary circuit.

Although there are differences in the level of hypothermia, the change of platelet count reported during hypothermic circulatory arrest7 is sometimes also found in newborn infants undergoing therapeutic hypothermia. That is, the platelet count decreases during therapeutic hypothermia and recovers after rewarming.

Here, mean platelet volume (MPV) is considered of value in adults as a predictor of cardiovascular disease, e.g., cerebrovascular disease or acute myocardial infarction. Recently, reports on the association between MPV and some diseases in neonates have also appeared. MPV is associated with platelet production, and it was thought that despite the occurrence of a time gap between MPV and platelet production, MPV could be used in the evaluation of platelet production.

In this study, we examined whether MPV and platelet count would change according to the use or nonuse of therapeutic hypothermia to treat neonatal hypoxic ischemic encephalopathy (HIE).

Subjects and Methods

The subjects included 128 infants who were diagnosed as having HIE among the newborn infants admitted to the NICU in this hospital between January 2013 and June 2015. We excluded infants with congenital anomaly (n=8, 6%), and among the infants receiving hyperthermia, those who could not undergo 72 hours of therapeutic hypothermia (n=7, 5%), those who were admitted after 24 hours after birth (n=8, 6%), those who died within 7 days after birth (n=4, 0.7%), and one infant (0.7%) transferred to another hospital within 7 days after birth in the TH group. In addition, we
excluded those tested on holidays and nights to eliminate differences in the measuring equipment (n=45, 35%). Thus, we examined 55 infants (42%) and divided them into the TH group, those who received therapeutic hypothermia (n=24), and the non-TH group, those who did not (n=31).

Items examined were sex, gestational age, birth weight, SD score, Apgar score (at 1 and at 5 minutes), mode of delivery, primipara or multipara, and platelet count and MPV, which were measured on admission, on postnatal day 5, and on postnatal day 7.

An automated multiple hematology analyzer XN-1000 (Sysmex Corporation) was used to measure MPV, and the platelet counts and MPVs in both groups were compared.

Statistical analyses were performed with Pearson’s product-moment correlation coefficient and the Mann-Whitney U test. The significance level was $p < 0.05$. The data were analyzed using statistical analysis software (IBM SPSS Statistics 20).

**Result**

The background of the subjects is shown in Table 1. One- and five-minute Apgar scores were both significantly low in the TH group, but no other significant differences were recognized in the other perinatal information.

The platelet count in the TH group decreased significantly from admission to postnatal day 5 ($p=0.04$) and increased from postnatal day 5 to postnatal day 7 ($p<0.01$) (Figure 1-a). In the non-TH group, no significant change was found from admission to postnatal day 5 ($p=0.52$), but the platelet count increased significantly from postnatal day 5 to postnatal day 7 ($p<0.01$). In comparing the two groups, the platelet count was high only on postnatal day 7 in the non-TH group ($p<0.01$).

MPV in the TH group increased significantly from admission to postnatal day 5 ($p=0.03$), but no significant difference was recognized from postnatal day 5 to postnatal day 7 ($p=0.36$) (Figure 1-b). In the non-TH group, MPV increased significantly from admission to postnatal day 5 ($p<0.01$), but no significant difference was recognized from postnatal day 5 to postnatal day 7 ($p=0.71$). In comparing two groups, MPV was high only on postnatal day 7 in the TH group ($p<0.01$).

No significant differences in platelet count and MPV were found in either the TH group or the non-TH group on admission and on postnatal day 5 (platelet count: $p=0.93$ on admission, $p=0.54$ on postnatal day 5; MPV: $p=0.54$ on admission, $p=0.51$ on postnatal day 5) (Table 2). However, on postnatal day 7, the platelet count was significantly lower ($p<0.01$) and the level of MPV was significantly higher ($p=0.03$) in the TH group.

In the TH group, a negative correlation was found in the relationship between platelet count and MPV on admission, on postnatal day 5, and on postnatal day 7 (admission: $p=0.02$, $R=0.46$; postnatal day 5: $p=0.03$, $R=0.43$; postnatal day 7: $p=0.03$, $R=0.42$) (Figure 2). In the non-TH group, however, a correlation was absent at all time points (admission: $p=0.93$, $R=0.11$; postnatal day 5: $p=0.07$, $R=0.28$; postnatal day 7: $p=0.58$, $R=0.13$).

**Table 1: Background of the subjects.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TH group (n=24)</th>
<th>non-TH group (n=31)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male infant (n)</td>
<td>12 (50%)</td>
<td>16 (51%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>39.2±1.3</td>
<td>39.3±1.4</td>
<td>0.56</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2933±332</td>
<td>3035±402</td>
<td>0.28</td>
</tr>
<tr>
<td>SD score</td>
<td>-0.14±0.98</td>
<td>0.07±0.92</td>
<td>0.54</td>
</tr>
<tr>
<td>Apgar score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 1 minute</td>
<td>2±2</td>
<td>4±1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>At 5 minutes</td>
<td>4±2</td>
<td>6±1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mode of delivery (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>7 (29%)</td>
<td>17 (55%)</td>
<td></td>
</tr>
<tr>
<td>Vaginal delivery with forced delivery</td>
<td>4 (17%)</td>
<td>4 (13%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>2 (8%)</td>
<td>2 (6%)</td>
<td></td>
</tr>
<tr>
<td>Urgent cesarean section</td>
<td>11 (46%)</td>
<td>8 (26%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Primipara (n)</td>
<td>11 (46%)</td>
<td>13 (41%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Sarnat classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>2 (8%)</td>
<td>22 (71%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Moderate</td>
<td>16 (67%)</td>
<td>8 (26%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Severe</td>
<td>6 (25%)</td>
<td>1 (3%)</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Table 2: Platelet count and MPV of both groups.

<table>
<thead>
<tr>
<th></th>
<th>TH group</th>
<th>Non-TH group</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelet count (× 10⁶/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On admission</td>
<td>24.0±7.0</td>
<td>24.4±5.2</td>
<td>0.93</td>
</tr>
<tr>
<td>On postnatal day 5</td>
<td>20.0±6.3</td>
<td>22.8±8.6</td>
<td>0.16</td>
</tr>
<tr>
<td>On postnatal day 7</td>
<td>31.7±13.1</td>
<td>41.1±6.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>MPV (fL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On admission</td>
<td>9.9±0.8</td>
<td>9.7±0.6</td>
<td>0.54</td>
</tr>
<tr>
<td>On postnatal day 5</td>
<td>10.6±1.2</td>
<td>10.2±0.5</td>
<td>0.51</td>
</tr>
<tr>
<td>On postnatal day 7</td>
<td>10.7±0.7</td>
<td>10.3±0.5</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Significant differences in platelet count and MPV were not recognized on admission and on postnatal day 5 between the two groups. However, on postnatal day 7, the platelet count was low, but the MPV level was high in TH group.

Fig. 1: a. Change in the platelet count. In the TH group, the platelet count decreased from admission to postnatal day 5 and increased from postnatal day 5 to day 7. In the non-TH group, no significant change in the platelet count was found from admission to postnatal day 5, but it increased from postnatal day 5 to day 7. b. Change in MPV. In the TH group, MPV increased from admission to postnatal day 5, but no significant difference was found from postnatal day 5 to day 7. In the non-TH group, MPV increased from admission to postnatal day 5, and no significant difference was found from postnatal day 5 to day 7.
Fig. 2: Relationship between platelet count and MPV. On admission: A negative correlation was found in the TH group (p=0.02, R=-0.46), but a correlation was absent in the non-TH group (p=0.93, R=0.11). On postnatal day 5: A negative correlation was found in TH group (p=0.03, R=-0.43), but a correlation was absent in the non-TH group (p=0.07, R=-0.28). On postnatal day 7: A negative correlation was found in the TH group (p=0.03, R=-0.42), but a correlation was absent in the non-TH group (p=0.58, R=-0.13).
Discussion

This study showed how the platelet count and MPV changed between the TH group and non-TH group. There were no significant differences in platelet count and MPV from admission to postnatal day 5 between the TH group and non-TH group. However, due to therapeutic hypothermia, the platelet count significantly decreased and high MPV levels were observed. In addition, the study was revealed that negative correlations between platelet count and MPV were found at all time points in the TH group.

Thrombocytopenia is defined as a platelet count of less than $150 \times 10^9/L$ and is recognized in 5% of healthy term infants. Furthermore, it was reported that 22 to 35% of the infants admitted to the NICU have decreased platelets. One of the most common thrombocytopenias that appears within 72 hours after birth is neonatal asphyxia. Infants with neonatal asphyxia leading to HIE receive therapeutic hypothermia under a situation of decreased platelet count, and this may cause a further decrease in the platelet count. In fact, the present results showed that the platelet count of the infants who received therapeutic hypothermia decreased more significantly than that in those who were not suitable for therapeutic hypothermia.

In general, the mechanism of thrombocytopoiesis is as shown in Figure 3. Immature platelets are larger than old platelets, and therefore a negative correlation exists between platelet count and MPV. Thus, the usefulness of MPV has been evaluated to differentiate whether thrombocytopenia is due to excessive destruction or underproductivity. That is, an elevated MPV level can be considered to be enhanced platelet production. Moreover, because even early after birth, an elevation of the MPV is caused by an increase of platelet production, it is reported that there is an association between elevated MPV level and increased platelet count in the neonatal period.

Furthermore, it is also reported that MPV is a marker of platelet production and consumption and may be related to bone marrow, hypoxia, and severity of diseases associated with perinatal inflammation and infection. Approximately 5 days are needed for platelet maturation, and therefore we thought that platelet maturation was mainly affected by the peripartum period until postnatal day 5, and after postnatal day 7, it was mainly affected by therapeutic hypothermia, and thus we compared the two time periods. Actually, Boutaybi et al. reported that in the therapeutic hypothermia group, the platelet count reached its minimum...
in the control group in terms of postnatal days. In the present study, there was a significant difference in the severity classification of HIE between the TH group and non-TH group, and it was thought that the difference was due to the effect of hypoxia in the peripartum period. From the viewpoint of the changes in platelet count and MPV, no difference was found until postnatal day 5. In other words, we could show no difference between the two groups in terms of an effect of HIE on platelet production. However, on postnatal day 7, significant differences in platelet count and MPV were found between the TH group and non-TH group. This was thought to be mainly due to the decrease in platelet count caused by the use of therapeutic hypothermia.

In addition, it is thought that sequestration of platelets during hypothermia and dissociation from the organs in which they accumulate and their reappearance during rewarming also contribute greatly. Thus, in the case of thrombocytopenia due to hypothermic circulatory arrest in adults, there is a report that the platelet count increases and then recovers during rewarming. In the present study, rewarming after therapeutic hypothermia in the newborn infants started 72 hours after birth, but the platelet count still had not recovered on postnatal day 7. We thought that compared with those of adults, the platelets of newborn infants needed more time to return to the circulating blood.

The principal site of sequestration during hypothermia is thought to be the liver. Moreover, morphological changes in chilled platelets have also been reported. Furthermore, severe bradycardia, hypotension, and static blood flow in the organs have been observed during cooling, and it is also reported that the “hypoperfusion” state enhances the adhesion of platelets to various tissue surfaces and the degree of stasis, and that as a result, temporary platelet aggregation and deposition occur in the areas with circulatory stasis.

For these reasons, circulating platelets are reduced even during therapeutic hypothermia. However, newborn infants who undergo therapeutic hypothermia are expected to be affected by severe bradycardia, hypotension, and organ blood flow before and after delivery, and we thought that these factors affected the platelet count. In fact, no difference in the platelet count was found between the TH group and non-TH group from admission to postnatal day 5. A significant increase in MPV was observed in both groups, and even if therapeutic hypothermia was performed early after birth, enhanced hemopoiesis presumably maintained the platelet count.

As one limitation in this study, there are differences in neonatal asphyxia and the severity of HIE between the two groups. The newborn infants receiving therapeutic hypothermia definitely had higher levels of severity than those not receiving therapeutic hypothermia. In this study, we observed changes in the platelet count to distinguish the effect before and after birth and the effect caused by therapeutic hypothermia. Additionally, the number of specimens was reduced because the measurement system used for blood testing on holidays and nights were different, and the results of those tests were excluded from the study. However, the number of specimens was not significantly different between the two groups.

We believe that in future, an increase in the number of cases examined will lead to a study that can contribute to prognostic prediction and treatment strategy.

**Conclusion**

We examined the changes in platelet count and MPV according to the use or nonuse of therapeutic hypothermia in newborn infants with HIE. In these infants, an effect on the cardiovascular system was recognized before and after delivery, but hematopoietic hyperactivity was thought to maintain the platelet count as it decreased. Furthermore, we surmised that even if therapeutic hypothermia was performed to treat a low platelet count early after birth, enhanced platelet production by bone marrow maintained the platelet count.

**References**


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