Chapter 4

Placental pathology and neurological morbidity in preterm infants during the first two weeks after birth

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Abstract:

Background: The placenta plays a crucial role during pregnancy and dysfunction causes long-term neurological problems. Identifying placenta-related risks for neurological problems shortly after birth may provide clues for early interventions aiming to improve neurological outcome.

Objective: To determine the association between placental pathology and neurological morbidity in preterm infants during the first two weeks after birth.

Study design: Placentas of 52 singleton, preterm infants (GA 25-31 weeks, BW 560-2250 grams) were examined for histopathology. Infants’ neurological condition shortly after birth was determined by assessing the quality of their general movements (GMs): normal, abnormal, or hypokinetic, on days 5, 8, and 15. A motor optimality score (MOS) was also assigned.

Results: Examination of the placentas revealed maternal vascular underperfusion (n=29), ascending intrauterine infection (AIUI) (n=19), villitis of unknown aetiology (n=6), chronic deciduitis (n=11), foetal thrombotic vasculopathy (FTV) (n=9), and elevated nucleated red blood cells (NRBCs) as a marker for foetal hypoxia (n=7). None of the placental lesions were significantly associated with the quality of GMs or MOS.

Conclusions: This study indicated that placental lesions were not associated with infants’ neurological condition as measured by the quality of their general movements during the first two weeks after birth.
Introduction

Preterm birth is one of the main causes of long-term neurodevelopmental problems in preterm infants. Placental pathology may act as a causative factor of preterm birth, with major implications for the child if placental function is impaired. A previous study suggested that several placental lesions are associated with early neonatal morbidity in preterm infants. We also know that several placental lesions are associated with long-term neurological morbidity. These lesions include ascending intrauterine infection, chronic villitis of unknown aetiology, meconium associated vascular necrosis, and foetal thrombotic vasculopathy. The appearance of elevated nucleated red blood cells, which is a marker for foetal hypoxia rather than a placental lesion, is also associated with long-term neurological morbidity. What we do not know, however, is whether these same placental lesions are associated with neurological morbidity in preterm infants shortly after birth. Placental dysfunction possibly has its greatest impact shortly after birth. A detailed understanding of the relation between placental lesions and an infant’s neurological condition shortly after birth is necessary to identify placenta-related risks for neurological problems and could provide clues for early interventions aiming to improve neurological outcome.

The most reliable method to evaluate the neurological condition of preterm infants shortly after birth is Prechtl’s method of assessing the quality of their general movements (GMs). In addition to the qualitative assessment of GMs, a semi-quantitative analysis of several qualitative aspects of GMs is expressed by a motor optimality score (MOS). The assessment of GMs is a sensitive and non-invasive method with high interobserver agreement (Kappa-value 0.88). GMs are predictive of neurological outcome.

Our objective was to determine whether placental pathology was associated with neurological morbidity in preterm infants during the first two weeks after birth as expressed by GM quality. We hypothesized that in the presence of placental pathology the quality of GMs of preterm infants is poorer and their MOS lower.

Methods

Patient population

Our cohort consisted of 57 preterm, singleton infants. All infants had been admitted to the Neonatal Intensive Care Unit of the Beatrix Children’s Hospital in Groningen, the Netherlands. The inclusion criteria were singleton infants with a gestational age (GA) of less than 32 weeks. Exclusion criteria were major chromosomal and congenital abnormalities. We also excluded infants whose placentas were not available for pathological examination (n=4) or if the video recordings to assess GMs on days 5, 8, and 15, were lacking (n=1). Our final study group consisted of 52 preterm singleton infants.

We recorded several clinical characteristics of the infants, including illness severity, based on the Score of Neonatal Acute Physiology Perinatal Extension (SNAPPE).
Placental pathology
The placentas were examined by a perinatal pathologist (AT) in accordance with the guidelines published by the Royal College of Obstetricians and Gynaecologists and the Royal College of Pathologists in Britain, and the College of American Pathologists. With the exception of GA, the pathologist was blinded as to clinical outcome. We included all the placental lesions for which an association with neurological impairment was suggested. The lesions were: placental pathology consistent with maternal vascular underperfusion (MVU), ascending intrauterine infection (AIUI), chronic villitis of unknown origin (VUE), chronic deciduitis, perivillous fibrinoid, foetal thrombotic vasculopathy (FTV), meconium associated vascular necrosis, chorioamniotic haemosiderosis, elevated nucleated red blood cells (NRBCs), chorangiosis, and umbilical cord abnormalities. Definitions and scoring criteria are presented in Table 1.
Table 1: Diagnostic terminology and definition of the placental lesions

<table>
<thead>
<tr>
<th>Diagnostic terminology</th>
<th>Definition and scoring criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal vascular underperfusion (MVU)</td>
<td>Decidual vasculopathy, e.g. incomplete or absent spiral artery remodelling, acute atherosis, fibrinoid necrosis or thrombosis; parenchymal pathology such as placental hypoplasia, increased syncytial knotting, villous agglutination, increased perivillous fibrin, distal villous hypoplasia, infarction, retroplacental hematomata.</td>
</tr>
<tr>
<td>Ascending intrauterine infection (AIUI)</td>
<td>Acute inflammation of the extraplacental membranes and chorionic plate. Acute chorioamnionitis and chorionitis represent the maternal response; chorionic or umbilical vasculitis represents the foetal response.</td>
</tr>
<tr>
<td>Villitis of unknown aetiology (VUE)</td>
<td>Chronic lymphohistiocytic inflammation of the stem- and chorionic villi, with or without obliterative vasculopathy of stem villous vessels.</td>
</tr>
<tr>
<td>Chronic deciduitis</td>
<td>Chronic lymphohistiocytic inflammation of the decidua.</td>
</tr>
<tr>
<td>Maternal floor infarction (MFI) / massive perivillous fibrinoid deposition (MPVFD)</td>
<td>Excessive perivillous fibrin deposition, either basally at a thickness of ≥3 mm on at least one slide (MFI) or transmural encasing ≥50% of villi on at least one slide (MPVFD).</td>
</tr>
<tr>
<td>Fetal thrombotic vasculopathy (FTV)</td>
<td>Fetal vascular thrombosis, intimal fibrin cushions, fibromuscular sclerosis, hemorrhagic endovasculitis and groups of at least 5 avascular fibrotic villi without inflammation or mineralization and/or adherent thrombi in stem vessels.</td>
</tr>
<tr>
<td>Meconium associated vascular necrosis</td>
<td>Meconium associated necrosis of smooth muscle cells in the wall of chorionic plate vessels.</td>
</tr>
<tr>
<td>Chorioamniotic haemosiderosis</td>
<td>Presence of hemosiderophages in the amnion and chorion.</td>
</tr>
<tr>
<td>Elevated nucleated red blood cells (NRBCs)</td>
<td>Only rare NRBCs are normal after the first trimester. More than an occasionally NRBC was considered as abnormal.</td>
</tr>
<tr>
<td>Chorangiosis</td>
<td>Diffuse increase in the number of villous capillaries.</td>
</tr>
<tr>
<td>Umbilical cord abnormalities</td>
<td>Obstruction or disruption of the umbilical cord blood flow (e.g. umbilical cord prolapse, entanglement, knots, disrupted velamentous vessels, hyper/hypo-coiling).</td>
</tr>
</tbody>
</table>

**Video recording of general movements**

We video recorded each infant’s general movements on days 5, 8, and 15. Each recording lasted 50 minutes. The infant lay supine in the incubator wearing only a nappy. We placed the video camera high above the infant at the foot of the incubator so as to obtain an unobstructed view of the infant’s entire body and face. The infant could move limbs and trunk freely. GMs during crying, hiccupping, or while the infant was sucking on a dummy were excluded from the analysis. 

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Analysis of general movements
AMR, MMH, and AFB assessed the quality of GMs according to Prechtl’s method. This method assesses the GMs on the basis of visual Gestalt perception. Normal GMs involve the infant’s entire body, can last a few seconds to several minutes, and are characterized by a complex and variable sequence of arms, legs, neck, and trunk. GMs are scored as abnormal if they lack complexity, variability, and fluency. There are three types of abnormal GMs that apply to the preterm period: poor repertoire, chaotic, and cramped-synchronized GMs. If GMs are absent or very short (<3 seconds), the infant was assessed as being hypokinetic.

A more detailed analysis of GMs is obtained by the motor optimality score (MOS) based on Prechtl’s optimality concept. For this purpose, we used a score sheet developed by Ferrari et al. and modified by De Vries et al. The highest, most optimal score is 18 and the lowest score is 8. A hypokinetic infant is assigned an 8. The assessors were blinded as to placental lesions.

Statistical analysis
MOS was taken as our primary outcome measure for calculating sample size. In a previous study the standard deviation of MOS was 3. With MOS ranging from 8 to 18, we considered a difference of 4.5 as relevant. We expected to find five large categories of placental lesions: MVU, AIUI, chronic deciduitis, FTV, and elevated NRBCs. On average 1 in 4 placentas are affected. We set the level of significance at α=0.01, applying the Bonferroni correction with regard to the five categories of placental lesions. With a power of 0.8 and a ratio of 1 to 4 with regard to the presence of placental lesions, we calculated that we needed to include at least 34 infants for the purpose of our study.

We used SPSS 20.0 software for Windows (SPSS Inc., Chicago, Illinois, USA) for the statistical analyses.
To analyze the course of infants’ GM quality and MOS during the study period, we used the chi-square test for trend and Spearman’s rho. We used the Wilcoxon signed rank test to analyze the course of the MOS for a specific placental lesion. For these tests $P<.05$ was considered statistically significant.

To test the association between pathological placental lesions and the quality of the GMs we used the chi-square test for trend. For the association with the MOS we used the Mann-Whitney test. In both cases we considered $P<.01$ statistically significant.

To analyze the association between placental weight and umbilical cord length and GM quality we used the students T-test and Mann-Whitney test, respectively. For the association with the MOS we used Pearson’s correlation and Spearman’s rank correlation coefficient, respectively.
Results

Patient characteristics

The patient characteristics are presented in Table 2. Two infants died between 9 and 12 days after birth of respiratory and circulatory failure due to sepsis.

Table 2. Patient characteristics. Data are given as median (range) or numbers.

<table>
<thead>
<tr>
<th>Study population</th>
<th>N=52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age in weeks</td>
<td>29.1 (25.1 – 31.7)</td>
</tr>
<tr>
<td>Birth weight in grams</td>
<td>1180 (560-2250)</td>
</tr>
<tr>
<td>Male/female</td>
<td>22/30</td>
</tr>
<tr>
<td>Apgar score 1 minute</td>
<td>6 (1-9)</td>
</tr>
<tr>
<td>Apgar score 5 minutes</td>
<td>8 (3-10)</td>
</tr>
<tr>
<td>Intracranial haemorrhage, grade 1-2</td>
<td>7</td>
</tr>
<tr>
<td>Intracranial haemorrhage, grade 3-4</td>
<td>4</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>6</td>
</tr>
</tbody>
</table>

Delivery characteristics

Spontaneous preterm birth       | 28            |

Induced

- foetal distress              | 15            |
- maternal reasons             | 4             |
- foetal and maternal reasons  | 5             |

Preterm pre-labour rupture of the membranes (PPROM) | 9             |

Caesarean section (elective and emergency) | 29            |

Placental weight in grams       | 250 (99 – 470) |

Cord length in centimetres      | 27 (15 – 59)  |
Placental pathology

In Table 3 we present the occurrence of placental pathologies, categorized by GA and birth weight (BW). Four out of the 52 placentas showed no pathology. Twenty-nine placentas showed pathology consistent with MVU (infarction, villous hypoplasia, retroplacental hematoma), 19 placentas had signs of AIUI (maternal response, foetal response or both). Thirty placentas showed more than one placental lesion. However, a combination of the two largest groups of lesions (MVU and AIUI) was only present in four placentas. Other combinations that were present in five placentas or more included MVU and elevated NRBCs (6 placentas), MVU and FTV (6 placentas), and MVU and chronic deciduitis (5 placentas). We found no placental lesions consistent with perivillous fibrinoid, meconium associated vascular necrosis, or complications of the umbilical cord.

Table 3. Presence of placental pathology specified by gestational age and birth weight.

<table>
<thead>
<tr>
<th>Placental lesions</th>
<th>&lt;28 wk</th>
<th>≥28 wk</th>
<th>&lt;1 kg</th>
<th>≥1 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVU (n=29)</td>
<td>6</td>
<td>23</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>AIUI (n=19)</td>
<td>8</td>
<td>11</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Chronic deciduitis (n=11)</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Chronic villitis (n=6)</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>FTV (n=9)</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Elevated NRBCs (n=7)</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Chorangiosis (n=2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chorioamniotic haemosiderosis (n=2)</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: MVU - maternal vascular underperfusion; AIUI - ascending intrauterine infection; FTV - foetal thrombotic vasculopathy; NRBCs - nucleated red blood cells.

The distribution of placental lesions specified by gestational age (<28 weeks and ≥28 weeks) and by birth weight (<1 kilo and ≥1 kilo). The numbers exceed totals, because a single placenta can have more than one lesion.
General movements
On day 5 we obtained videos of 46 infants, on day 8 of 43 infants, and on day 15 of 43 infants. We observed normal, poor repertoire and chaotic GMs, and hypokinesia. The number of infants with normal GMs increased significantly during the study period (chi-square test for trend, $P=.04$). The MOS score tended to increase with increasing age (Spearman’s rho, 0.16, $P=.07$).

Placental pathology and general movements
The number of placental lesions did not correlate with GM quality. We present the presence or absence of specific placental lesions in relation to the quality of GMs on days 5, 8, and 15 in Table 4. On day 5, more infants had abnormal GMs in the presence of elevated NRBCs and FTV but it just failed to reach significance (chi-square test for trend, $P=.06$ and $P=.06$, respectively). On day 5, none of the placental lesions were associated with the MOS. On day 8 and 15, none of the placental lesions were associated with the quality of GMs or with the MOS. On days 5, 8 and 15 placental weight was associated with MOS ($r=0.35$ $P=0.02$, $r=0.37$ $P=0.02$ and $r=0.38$ $P=0.01$, respectively) and with the quality of GMs ($P=0.03$, $P=0.01$, and $P=0.01$, respectively). Lower placental weight was associated with lower MOS and more abnormal GMs. Umbilical cord length was only on day 8 associated with GM quality ($P=0.02$). Shorter umbilical cord length was associated with more abnormal GMs.
Table 4: Relation between placental lesions and the quality of GMs on days 5, 8 and 15.

<table>
<thead>
<tr>
<th></th>
<th>Day 5 n=46</th>
<th>Day 8 n=43</th>
<th>Day 15 n=43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVU Present</td>
<td>1</td>
<td>2</td>
<td>0.76</td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
<td>2</td>
<td>0.18</td>
</tr>
<tr>
<td>AIUI Present</td>
<td>0</td>
<td>1</td>
<td>0.10</td>
</tr>
<tr>
<td>Absent</td>
<td>1</td>
<td>3</td>
<td>0.49</td>
</tr>
<tr>
<td>Chronic</td>
<td>0</td>
<td>6</td>
<td>0.22</td>
</tr>
<tr>
<td>deciduitis</td>
<td>1</td>
<td>21</td>
<td>0.06</td>
</tr>
<tr>
<td>VUE Present</td>
<td>1</td>
<td>25</td>
<td>0.06</td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
<td>16</td>
<td>0.06</td>
</tr>
<tr>
<td>FTV Present</td>
<td>1</td>
<td>23</td>
<td>0.06</td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
<td>23</td>
<td>0.06</td>
</tr>
<tr>
<td>↑NRBCs Present</td>
<td>1</td>
<td>4</td>
<td>0.06</td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
<td>4</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Abbreviations: GMs - general movements; H - hypokinetic; CH - chaotic; CS - cramped synchronized; PR - poor repertoire; N - normal; MVU - maternal vascular underperfusion; AIUI - ascending intrauterine infection; VUE - villitis of unknown aetiology; FTV - foetal thrombotic vasculopathy; NRBCs - nucleated red blood cells.
Potential confounders or mediators of GM quality
We performed unifactorial analyses to determine whether GM quality was affected by other clinical characteristics. Lower gestational age and birth weight were both associated with more abnormal GMs and lower optimality scores on day 5, 8 and 15 (Mann-Whitney test and Spearman's rho \( P<0.01 \)). A higher illness severity during the first 24 hours after birth as determined using the SNAPPE score was associated with lower optimality scores on all days (Spearman's Rho \( P<0.01 \)). Intubation after one week was also associated with more abnormal GMs (chi-square test for trend, \( P<0.01 \)). Being small or appropriate for gestational age and intracranial haemorrhages (mostly grade 1-2) were not associated with the quality of GMs.

Next, we checked whether these potential confounders were also related to placental lesions. A lower birth weight was associated with a higher occurrence of FTV and elevated NRBCs (Mann-Whitney test \( P<0.05 \)). A higher illness severity during the first 24 hours after birth was associated with both FTV and elevated NRBCs (Mann-Whitney test \( P<0.05 \)). We did not find an association between any placental lesion and respiratory conditions requiring intubation or additive oxygen after one week, or between placental lesions and intracranial haemorrhages.

Discussion
This study indicated that placental lesions were not associated with infants' neurological condition as measured by the quality of their general movements during the first two weeks after birth. We hypothesized that placental dysfunction had its greatest impact shortly after birth. This was not confirmed in our study. Only placental weight was associated with the quality of GMs on all days.

We assessed GMs during the first two weeks after birth. It might well be that during this period the quality of GMs is not fully reflected by the placental lesions, because other clinical and biochemical factors influence the quality of GMs more during this period. This might especially be true for the preterm period. Many physiological changes take place during the first weeks after birth that influence brain function and, as a consequence, the quality of GMs.\(^7\) In addition, very preterm infants often have complicating illnesses that may affect brain function in the acute phases. In our study we found lower gestational age, lower birth weight, higher illness severity and ventilatory support to be associated with poorer GM quality. It might be, however, that lower gestational age confounded the relations of illness severity and ventilatory support to GM quality. The relation between clinical factors and GM quality suggests that other factors had more influence on GM quality than placental pathology shortly after birth. It might be that effects of placental lesions on neurological functioning in preterm infants become apparent only later in life.

Placental pathology consistent with FTV and elevated NRBCs (a marker for foetal hypoxia) nearly reached significance with abnormal GM quality. These lesions were associated with high illness severity, which confirms findings in a previous study.\(^2\) In the present study, high illness severity was also associated with abnormal GM quality.
This higher illness severity might, in turn, have affected GM quality explaining the near significant associations between these lesions and GM quality. This is, however, highly speculative.

In term infants, FTV and elevated NRBCs are highly associated with neurological impairment and cerebral palsy. FTV is also associated with a higher incidence of obstetric and perinatal complications and an increase in foetal cardiac abnormalities. In addition to the explanation stated above, another reason that the association between elevated NRBCs and the quality of GMs in our preterm cohort just failed to reach significance might be due to the small number of placentas with this lesion in our group (7 out of 52). We considered a difference of 4.5 points on the motor optimality score relevant, with these numbers the power for elevated NRBCs was 64%. However, the power of FTV was sufficient (82%). Despite the marginal power of elevated NRBCs, the borderline significance of FTV and elevated NRBCs might be relevant and important. Our results suggest that these particular lesions are associated with neurological impairment, not only in term infants but also in preterm infants.

MVU was reported to occur more often in intrauterine foetal deaths, especially between 24 and 32 weeks of gestation. In infants >35 weeks GA, it was suggested that macroscopic placental infarcts are associated with an increased risk of cerebral palsy. A study in term infants, however, showed no association between MVU and neurological impairment. Our present findings in live-born preterm infants were consistent with this study. MVU was present in 29 out of the 52 placentas, with this number we should have been able to find a difference if it was present (power 99.6%).

Previous studies showed that AIUI, especially in the presence of a high grade foetal response, is associated with neurological impairment at the age of two. Others associate it with cerebral palsy and abnormal neurocognitive function at school age, possibly due to elevated cytokines and cardiovascular instability. In our study we did not grade the foetal response, which might possibly explain why we did not find an association between AIUI and neurological impairment. AIUI is also suggested to be associated with lower Apgar scores at 1 and 5 minutes after birth. In a previous study we found no association between AIUI and illness severity in the first 24 hours after birth. This is consistent with this study were we also found no association between AIUI and the quality of GMs. Again the power would have been sufficient to demonstrate differences if present (99%).

In this study we did find an association between placental weight and quality of GMs. On days 5, 8 and 15 lower placental weight was associated with more abnormal GMs and lower MOS. Again, it might be that lower gestational age confounded this relation between placental weight and GM quality. In literature, findings relating placental weight and adverse neurological outcomes are inconclusive. Both light and heavy placental weight is suggested to be associated with adverse neurological outcome. Low placental weight is also suggested to be associated with stillbirth. Our findings indicate that low placental weight is also associated with early neurological morbidity.

We acknowledge several limitations of our study. Firstly, we only included singletons.
so as to be certain that each infant was linked to its own placenta. In twins placental pathology is probably different, e.g. twin-to-twin transfusion. Secondly, we did not determine the quality of GMs in our study group with such rare placental lesions as diffuse villous oedema and/or recent non-occlusive chorionic vessel thrombi in association with chorioamnionitis. In an earlier study, such lesions were found to be associated with adverse neurological outcomes. Thirdly, several lesions we focused on, i.e. chronic villitis, foetal thrombotic vasculopathy, elevated NRBCs, chorangiosis, and mecoinium associated vascular necrosis, are lesions found in term placentas. This might be a reason why these lesions were present in only a few preterm placentas. Finally, only four children in our group had no placental lesions. Almost all children had one or more placental lesion. When determining associations between placental lesions and GM quality, the comparison group partly consisted of infants with other placental lesions than the one studied.

In conclusion, FTV and elevated NRBCs showed a borderline association with the quality of GMs. Other placental lesions were not associated with the quality of GMs. We demonstrated that it is difficult to identify a placenta-related risk group for neurological problems as measured by the quality of GMs shortly after birth. This might be because other conditions related to preterm birth might confound a possible association between placental lesions and the quality of GMs.

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PLACENTAL PATHOLOGY AND NEUROLOGICAL MORBIDITY IN PRETERM INFANTS
DURING THE FIRST TWO WEEKS AFTER BIRTH
Chapter 5: Placental lesions and neurodevelopmental outcome at toddler age

Chapter 6: The relation between placental lesions and functional outcomes at early school age of children born between 32 and 36 weeks' gestational age