Clinical Case Study of ABO Hemolytic Disease in Full-term Newborns with Positive Free Test and Direct Antiglobulin Test

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Keywords: Newborn, ABO Hemolytic Disease, Free Antibody Test, Free Antibody Test, Direct Antiglobulin Test.

Abstract: ABO hemolytic disease of newborn (ABO-HDN) occurs early and progresses rapidly, which can lead to fetal or neonatal anemia and neonatal hyperbilirubinemia. Severe intrauterine hemolysis can lead to fetal edema, which can lead to fetal death or neonatal death. Severe or very severe hyperbilirubinemia can be complicated with bilirubin encephalopathy, Affect the quality of life of newborns. In this study, full-term children with ABO-HDN were divided into two groups according to the results of three serological tests of hemolysis. Group A: single antibody release test (ART) was positive; Group B: ART positive, combined with at least one hemolysis test positive in direct antiglobulin test (DAT) and free antibody test (FAT). The differences of minimum hemoglobin, age at onset (h) and incidence of anemia between group A and group B were compared.

1 INTRODUCTION

ABO-HDN postpartum diagnosis methods mainly rely on three hemolysis tests, as follows: ① FAT is one of the necessary detection methods for the postpartum diagnosis of ABO-HDN. It can check whether there is free IgG antibody in the serum of the newborn and indirectly indicate the concentration of antibody in the serum of the child. It has guiding significance for the hemolysis persistence of the child and the clinical diagnosis and treatment of ABO-HDN and the progress of the disease. In this test, adult red blood cells of the same blood group are dropped into the serum of sick newborns, and then anti human globulin serum is dropped. Red blood cell agglutination indicates that the test is positive. ② DAT is a basic method for the diagnosis of ABO-HDN. Positive enhancement of DAT is correlated with the demand for phototherapy, and no false positive DAT cases have been found (Valsami, Politou, Boutsikou, et al. 2015). DAT is a good indicator for predicting the development of ABO-HDN with ABO incompatibility (Aydin, Deveci, Orman, et al. 2016, Ulrich, Ellsworth, Carey 2014). If there is IgG antibody inconsistent with erythrocyte membrane surface antigen in newborns, it can combine with erythrocyte membrane surface antigen to form immune complex. However, due to the small molecular weight of incomplete antibody, it can not connect the antigen antibody complex well, which only leads to the sensitization of red blood cells. After the anti globulin serum is added, it can bind with the incomplete antibody adsorbed on the red blood cells, so as to link the sensitized red blood cells and produce visible agglutination. Direct antiglobulin test (DAT) can detect the presence of IgG anti-A or anti-B and C3 on erythrocyte membrane to help identify hemolysis as immune dependent or immune independent. In the test, EDTA is best used for blood samples (Shaz, Hillyer, Gil 2019). Meanwhile, the neonatal DAT results can well predict their compliance with the therapeutic indication of hyperbilirubinemia (Shi, Ma, Zhu, et al. 2018). ③ ART uses the reversibility of antigen antibody reaction to release IgG antibody on sensitized red blood cells of children with neonatal hemolytic disease into normal saline through physical or chemical methods. The released IgG antibody still has biological activity. Standard type A or type B red blood cells are added to the release solution, and the released IgG antibody will sensitize red blood cells, Subsequently, anti human globulin serum was added, and the red blood cell aggregation visible to the naked eye was positive. Among the three detection methods

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of abo-hdn hemolysis, the positive rate of antibody release test is the highest (Daniel 2019). The combination of heat release test and acid release test can improve the positive detection rate of neonatal ABO hemolytic disease and reduce the risk of missed detection in release test (Chen, Deng, Huang, et al. 2019). Compare the results of slide test, tube test and microcolumn gel test. Results the success rate of ABO blood group identification by microcolumn gel method was higher than that of slide method + test tube method (You 2019).

Microcolumn gel cassette detection (MGCP) is highly sensitive to serological tests of hemolytic disease of the newborn by MGCP. It can significantly improve the detection rate of ABO-HDN positive, which can effectively reduce the missed diagnosis rate. Moreover, the MGCP test is convenient and quick, and easy to operate. It is better than the three test of ABO-HDN in vitro with the test tube anti human globulin test (TAT). Some studies also found that compared with venous blood test, umbilical cord blood hemolysis serological test is helpful to early diagnose neonatal ABO-HDN and control the disease as soon as possible (Hu, Zhang 2019). This study retrospectively analyzed the clinical data of children with ABO-HDN who met the diagnosis and treatment criteria, and discussed the clinical manifestations and influence of full-term ABO-HDN with positive free test and direct antiglobulin test.

2 SUBJECTS AND METHODS

2.1 Study Subjects

The subjects selected 127 term neonates who met the diagnostic criteria for the ABO-HDN.

2.2 Inclusion Criteria

According to the diagnostic criteria in practical neonatology (4th Edition) for ABO-HDN of the newborn, the diagnosis of ABO-HDN was confirmed based on maternal and child ABO blood type (mother with blood type O, child with blood type A or B, and both mother and child with Rh blood type positive), jaundice, and positive serological tests (DAT positive or ART positive); Received phototherapy, intravenous human immunoglobulin therapy; Condition improved discharge; No specific treatment was given out of hospital.

2.3 Exclusion Criteria

(1) Combined RH hemolytic disease; (2) Preterm infants; (3) Children discharged automatically, unable to implement treatment normally; (4) Readmitted children; (5) Combined G-6-PD deficiency; (6) Combined severe neonatal asphyxia; (7) Complicated neonatal sepsis; (8) Children with clinical symptoms of fever; (9) Comorbid congenital heart disease.

2.4 Research Methods

SPSS statistics 16.0 software was used for statistical analysis. The counting data were described by the number of cases and percentage (n, %). The measurement data were described by mean±standard deviation (X±S), and the differences between groups were compared by independent sample t-test, analysis of variance and chi square test; According to the results of correlation factor analysis, the factors with P<0.05 were included in logistic regression analysis, and P<0.05 was statistically significant.

3 COMPARISON OF EFFECTS OF DIFFERENT HEMOLYSIS TEST GROUPS ON MINIMUM HEMOGLOBIN, AGE AT ONSET (H) AND INCIDENCE OF ANEMIA

In this group of 127 cases, except ART positive, other results of hemolysis tests were DAT positive in 18 cases (14.2%) and FAT positive in 64 cases (50.4%) (see Table 1).

According to the different results of three serological tests of hemolysis, they were divided into two groups. Group A: ART positive alone; Group B: ART positive, combined with at least one hemolysis test of FAT and DAT positive. Independent sample t-test was used between group A and group B to compare the differences of minimum hemoglobin and age at onset (h), and chi square test was used to compare the differences of anemia incidence. The results are shown in Table 2.

The lowest hemoglobin in group B was significantly lower than that in group A (P<0.05), the age at onset (h) was earlier than that in group A, the difference was statistically significant (P=0.052), and the incidence of anemia was higher than that in group A (P<0.05).
Table 1: Results of the three hemolysis tests in group A and group B.

<table>
<thead>
<tr>
<th>class,(n)</th>
<th>ART positive</th>
<th>FAT positive</th>
<th>DAT positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (n=59)</td>
<td>59</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group B (n=68)</td>
<td>68</td>
<td>64</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 2: The results of different hemolysis tests were compared between the lowest hemoglobin, age (h) and anemia.

<table>
<thead>
<tr>
<th>class (n)</th>
<th>Minimum Hb (g/L)</th>
<th>Age at disease onset (h)</th>
<th>Anemia incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X ± s</td>
<td>X ± s</td>
<td></td>
</tr>
<tr>
<td>Group A (n=59)</td>
<td>147.39 ± 18.04</td>
<td>27.44 ± 14.70</td>
<td>40.7%</td>
</tr>
<tr>
<td>Group B (n=68)</td>
<td>130.74 ± 20.66</td>
<td>21.75 ± 17.53</td>
<td>72.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>/2value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>0.052</td>
</tr>
</tbody>
</table>

Note: * Use the chi-square test; 1 x refers to the mean, s refers to the standard deviation; 2 The denominator of the incidence of anaemia was 127 term ABO-HDN cases enrolled.

4. THE INFLUENCING FACTORS OF MODERATE AND SEVERE ANEMIA WERE ANALYZED BY BINARY LOGISTIC REGRESSION

4.1 Univariate Analysis of Moderate and Severe Anemia in ABO-HDN

A total of 127 full-term ABO-HDN cases included in the study were subjected to univariate analysis based on the occurrence or absence of moderate to severe anemia, using different hemolysis serology results as independent variables, and the relevant assignments are presented in table 3.

<table>
<thead>
<tr>
<th>project</th>
<th>classify</th>
</tr>
</thead>
<tbody>
<tr>
<td>No moderate or severe anemia occurred (n=104)</td>
<td>Moderate and severe anemia occurred (n=23)</td>
</tr>
<tr>
<td>Group B</td>
<td>49</td>
</tr>
<tr>
<td>y2value</td>
<td>9.54</td>
</tr>
<tr>
<td>P value</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Note: * P<0.05 has statistical differences

4.2 Binary Logistic Regression Analysis of Moderate and Severe Anemia in ABO-HDN

Table 4 shows that group B with ART positive and at least one positive hemolysis test result of DAT and FAT is P= 0.002, which is statistically significant (P < 0.05). It is included in the binary logistic regression analysis model, P < 0.05 is statistically significant, and the results are shown in Table 5.

The results in Table 5 showed that the incidence of moderate to severe anemia in ABO-HDN patients with ART positive and at least one positive for DAT and FAT was 5.33 times higher than that of ABO-HDN patients with ART positive alone.

Table 5: Binary logistic regression analysis of related factors of moderate and severe anemia in ABO-HDN.

<table>
<thead>
<tr>
<th>analytical factor</th>
<th>B</th>
<th>S.E.</th>
<th>Wals</th>
<th>P</th>
<th>OR</th>
<th>OR of 95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysis test results group B</td>
<td>1.67</td>
<td>0.58</td>
<td>8.21</td>
<td>0.004</td>
<td>5.33</td>
<td>1.70</td>
</tr>
</tbody>
</table>

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According to the results of logistic regression, the ROC curve was drawn. With the occurrence of moderate and severe anemia as the reference, the area under the ROC curve (AUC) of group B was 0.677, indicating that there was a certain reference value for predicting the occurrence of moderate and severe anemia according to the results of hemolysis test. The sensitivity of clinical prediction was 82.6% and the specificity was 52.9%. It shows that the model has a high accuracy in predicting the occurrence of moderate and severe anemia (see Figure 1).

![ROC Curve](image)

Figure 1: Logistic ROC Curve of regression Model.

5 CONCLUSIONS

In this study, the results of three postpartum hemolysis tests for term ABO-HDN were divided into two groups, A and B, to explore whether term ABO-HDN with different positive serological test results (ART, FAT, DAT) had differences. The results showed that patients with ART positive, combined with FAT and DAT at least one hemolysis test positive group had significantly lower lowest hemoglobin than the ART positive group alone, and the results were statistically significant (P<0.05). It was significantly earlier than the cases in the ART positive group alone, and the result was statistically significant (P < 0.05), and the incidence of anemia was significantly higher than that in the ART positive group alone (P < 0.05).

In the logistic regression analysis of the risk factors for predicting the occurrence of moderate and severe anemia, the case group with positive ART and at least one of DAT and FAT was positive, compared with the ART positive group alone, the risk of moderate and severe anemia increased by 5.33 times. The results were statistically significant (P<0.05).DAT is a confirmed test of neonatal ABO hemolysis, which has a lower positive rate in ABO than in RH hemolysis. Although FAT cannot be used as a decisive criterion for the diagnosis of neonatal ABO hemolysis, it can detect whether there are free IgG antibodies in neonatal serum and indirectly reflect the degree and persistence of ABO, which is of guiding significance for the clinical diagnosis and treatment of ABO-HDN and disease progress. Clinically, children with positive ABO-HDN combined with at least one positive DAT and FAT should be actively treated, so as to minimize the incidence of children complicated with anaemia, severe hyperbilirubinemia, and bilirubin encephalopathy. In conclusion, in full-term ABO-HDN cases, the serological hemolysis test was positive for ART, combined with DAT and at least one positive case with an increased moderate and severe risk of FAT anemia.

REFERENCES


Valsami S, Politou M, Boutsikou T. (2015), et al. Importance of Direct Antiglobulin Test (DAT) in Cord