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# A case of severe haemolytic disease of the newborn due to anti-Di<sup>a</sup> antibody

## 抗Di<sup>a</sup>抗體引起新生兒嚴重溶血症的病例

Red cell allo-antibodies directed against the Diego (Di) blood group antigen have rarely been reported to cause a haemolytic reaction against transfusion or haemolytic disease of the newborn. The frequency of the Di(a+) phenotype among the Hong Kong Chinese population is estimated to be 4.4%. We report on a case of severe haemolytic disease of the newborn due to anti-Di<sup>a</sup> antibody—the first local case to the best of our knowledge. Rare but clinically significant antibodies targeting red blood cells have to be considered in the investigation of haemolytic disease of the newborn when common underlying factors have been eliminated.

紅血球異體抗體與迪戈血型抗原產生對抗，引起溶血反應或導致新生兒患上溶血症，是非常罕見的病例。據估計，Di(a+)表型在香港華人口中出現的比例為4.4%。本文描述一個新生兒因抗Di<sup>a</sup>抗體引致嚴重溶血症，就我們所知，這是香港首宗同類病例。在診斷新生兒溶血症的過程中，如果發現病症並非由一些常見的基本病因造成，就必須考慮是否由一些罕見但在臨床上非常重要的抗體侵襲紅血球而引起的。

### Introduction

The role of anti-Di<sup>a</sup> antibody in causing haemolytic disease of newborn was first recognised in 1955.<sup>1</sup> Genetic studies reveal that there is great variation in the distribution of the Di<sup>a</sup> antigen in different populations. It is relatively common among Asian and South Americans, as compared with Caucasians.<sup>2</sup> Here we report the first local case of severe haemolytic disease of the newborn due to anti-Di<sup>a</sup> antibody.

### Case report

A baby girl was born at 38 weeks of gestation by vaginal delivery. She was the second child of a non-consanguineous couple, whose first child was healthy. The antenatal course was unremarkable. Routine screening for allo-antibodies against red blood cells at the first antenatal visit gave negative results. The baby's birthweight was 3105 g and the placenta weighed 750 g. Meconium-stained liquor was noted at birth. Physical examination revealed a pale and jaundiced baby. The liver and spleen were enlarged to 4 cm and 2 cm below the costal margin, respectively. There was no hydropic change and no evidence of acute haemorrhage.

Initial investigations at birth showed that the haemoglobin level was 59 g/L, the white blood cell count (corrected for nucleated red blood cells) was  $26.3 \times 10^9$  /L, and the platelet count was  $76 \times 10^9$  /L. The peripheral blood film showed a leuko-erythroblastic picture and many circulating nucleated red blood cells (170 per 100 white blood cells), as well as polychromasia and the presence of spherocytes. Furthermore, there was significant reticulocytosis (27% of red cell count). The serum haptoglobin level was low, at less than 0.05 g/L (reference range, 0.16-1.97 g/L), whereas the whole-blood methaemalbumin level was high, at 27 mg/L (reference level, <1 mg/L). Liver function tests showed predominant unconjugated hyperbilirubinaemia. The total serum bilirubin level at 3 hours of life was 186  $\mu$ mol/L (threshold for exchange transfusion was approximately 130  $\mu$ mol/L).

#### Key words:

*Erythroblastosis, fetal;*  
*Infant, newborn*

#### 關鍵詞：

成紅細胞增生症，胎兒；  
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Serial investigations were conducted to determine the cause of severe anaemia and haemolysis. Tests for cord-blood glucose-6-phosphate dehydrogenase level and thyroid function gave normal results. Investigations to detect sepsis, including blood cultures and surface swabs, yielded no organisms. Screening for congenital viral infection, including rubella and toxoplasmosis, also gave negative results. Autoimmune markers and immunoglobulin (Ig) levels were unremarkable. Parvovirus IgM was absent. Haemoglobin pattern analysis showed no significant abnormality, and a Heinz body preparation was negative.

Direct Coombs' testing on foetal red blood cells was strongly positive, both for polyspecific anti-human globulin and anti-IgG. The maternal blood was group B, Rh D-positive and the baby's blood was group AB, Rh D-positive. Maternal red blood cells gave negative results in the direct Coombs' test. No antibodies against red blood cells were identified in tests using commercial screen cells (DiaCell; DiaMed AG, 1785 Cressier s/Morat, Switzerland), and two screen cells from the Hong Kong Red Cross Blood Transfusion Service. The screen cells from both sources expressed the most frequently occurring and important red blood cell antigens, including  $Mi^a$ , but they did not express  $Di^a$ . However, compatibility testing during preparation for exchange transfusion showed that, among eight blood units selected, one was incompatible with maternal serum. An irregular antibody was thus suspected, and was indeed identified in the maternal serum from tests with an extensive panel of red blood cells: the specificity of the antibody was subsequently characterised as anti- $Di^a$ . Maternal red blood cells were then found to be  $Di(a)$ -negative, whereas red blood cells of the baby and her father were  $Di(a)$ -positive. The incompatible blood unit was also typed to be  $Di(a+b+)$ . The overall picture was consistent with severe haemolytic disease of the newborn due to an anti- $Di^a$  antibody. Elution studies using foetal red blood cells were not performed to confirm the identity of the antibody, because exchange transfusion was urgently needed and the pretransfusion blood sample was inadequate for study.

The serum bilirubin level increased to 270  $\mu\text{mol/L}$  at 8 hours of life despite intensive phototherapy. Double-volume exchange transfusion (ie using twice the estimated blood volume of the baby) was performed with group B and group O, Rh-positive whole blood. The bilirubin level dropped to an acceptable range only after three double-volume exchange transfusions. A total of 1165 mL fresh whole blood was used. The baby's condition remained stable after exchange transfusion. She was ultimately discharged on day 8 of life. Clinical follow-up until 2 years of age showed no residual anaemia or abnormal neurodevelopment. The brainstem-evoked potential at 3 months was within the normal range. Repeated blood counts and liver function test results were all unremarkable. The most recent blood counts, at 18 months, were as follows: haemoglobin, 136 g/L; white blood cell count,  $12 \times 10^9 /\text{L}$ ; and platelet count,  $266 \times 10^9 /\text{L}$ .

## Discussion

Blood group antigens comprise a wide range of molecules that have various biological functions on the erythrocyte membrane. The  $Di$  blood group system consists of nine antigens,<sup>3</sup> including two independent pairs of alleles:  $Di^a/Di^b$  and  $Wr^a/Wr^b$ . They belong to one of the three red blood cell membrane transporters<sup>4</sup> that are encoded by a gene on chromosome 17q12-q21.<sup>5</sup> The three red blood cell membrane transporters are called band 3 ( $Di$  system antigen), aquaporin 1 (Colton antigen), and HUT 11 (Kidd antigen). The  $Di$  system antigen acts as an anion exchanger that permits bicarbonate (hydrogencarbonate) ions to cross the membrane in exchange for chloride ions. The transport of bicarbonate ions in the plasma thus greatly increases the quantity of carbon dioxide that the blood can convey to the lungs.<sup>4</sup> This antigen also helps maintain the structural integrity of the erythrocyte membrane by stabilising membrane lipids.<sup>6</sup> It may also be involved in the removal of senescent red cells from the circulation.<sup>6</sup>

**Table. Summary of reports of anti- $Di^a$  antibody causing haemolytic disease of newborn**

Study	Nationality of mother	No. of cases	Features
Layrisse et al, <sup>1</sup> 1955	Venezuelan	1	First reported case
Alves de Lima et al, <sup>7</sup> 1982	Japanese	1	Severe HDN* requiring exchange transfusion
Goto et al, <sup>8</sup> 1987	Japanese	3	3 cases of mild HDN requiring phototherapy only
Peng et al, <sup>9</sup> 1996	Chinese	1	Severe HDN requiring exchange transfusion

\* HDN haemolytic disease of the newborn

The anti- $Di^a$  antibodies are polyclonal IgGs of the subclass IgG1 and IgG3. Unlike other antibodies of this Ig subclass, anti- $Di^a$  antibodies are unable to trigger complement activation. They can, however, induce macrophage phagocytosis of sensitised red blood cells.<sup>7</sup> Anti- $Di^a$  antibody was first recognised when it caused haemolytic disease in a baby born to a Venezuelan woman in 1955.<sup>1</sup> Several similar cases were subsequently described in Asia (Table).<sup>1,7-9</sup> Some, but not all, cases were severe enough to require exchange transfusion.<sup>9</sup> The reason for the variation in clinical severity of anti- $Di^a$  antibody-induced haemolytic disease of the newborn remains unknown but, as in cases of the disease caused by other red blood cell antibodies, the degree of haemolysis may depend on the maternal antibody concentration, the IgG subclass of the antibody, and the biological activity of the antibody as determined from cellular bioassays. Another potential modifying factor is the presence of maternal antibodies that can block the Fc-receptor and which can interfere with the binding of IgG-coated red blood cells to foetal macrophages, thereby reducing or preventing the haemolytic process. This situation has been shown in haemolytic disease of the newborn related to anti-D antibody.<sup>10</sup> There is no known case of hy-

drops foetalis related to the Di blood group–incompatible couples reported so far.

The main importance of the Di blood group is in anthropology, rather than haematology, because of its distinct racial distribution. The Di<sup>a</sup> antigen is very rare among Caucasians, but is relatively common (5%-15%) among South American Indians and Asian populations.<sup>2</sup> In a study of the distribution of various blood group antigens in Taiwan, the frequency of the Di<sup>a</sup> antigen among different Chinese ethnic groups was as follows: Minnan, 3.2%; Hakka, 7.0%; descendants of mainland Chinese from the south of Yangtse River, 3.2%; and descendants of mainland Chinese from the north of Yangtse, 10.3%.<sup>11</sup> From Chinese history, we know that the Hakka migrated to South China hundreds of years ago from northern China. The Hakka bloodline has stayed relatively pure, without much mixing with the indigenous people of the southeastern coastal area of China. Some researchers believe that Japanese descendants came from northern China. It is thus not surprising that the frequency of the Di<sup>a</sup> antigen among Hakka, northern Chinese, and Japanese populations is quite similar. The reported Di<sup>a</sup> antigen frequency in Hong Kong and Japan are 4.4% and 9.3%, respectively.<sup>12</sup>

## Conclusion

Given that the reported Di<sup>a+</sup> antigen frequency in Hong Kong is 4.4%, and that antibodies directed against the Di blood group system are clinically significant, it is worthwhile to consider anti-Di<sup>a</sup> antibody as a cause of haemolytic disease of the newborn when more common red blood cell antibodies are excluded. When red blood cells aimed at

testing against a Di<sup>a+</sup> panel are not immediately available, a compatibility test between paternal red blood cells against maternal serum, provided that they are ABO-compatible, may reveal the presence of uncommon antibodies in the maternal circulation.

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