

## CASE REPORT

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### HEMOLYTIC DISEASE OF THE NEWBORN DUE TO ANTI-U

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Anti-U is a rare red blood cell alloantibody that has been found exclusively in blacks. It can cause hemolytic disease of the newborn and hemolytic transfusion reactions.

We describe the case of a female newborn presenting a strongly positive direct antiglobulin test due to an IgG antibody in cord blood. Anti-U was recovered from cord blood using acid eluate technique. Her mother presented positive screening of antibodies with anti-U identified at delivery. It was of IgG1 and IgG3 subclasses and showed a titer of 32. Monocyte monolayer assay showed moderate interaction of Fc receptors with maternal serum with a positive result (3.1%). The newborn was treated only with 48 hours of phototherapy for mild hemolytic disease. She recovered well and was discharged on the 4<sup>th</sup> day of life.

We conclude that whenever an antibody against a high frequency erythrocyte antigen is identified in brown and black pregnant women, anti-U must be investigated.

**DESCRIPTORS: Anti-U. Antibody titration. Hemolytic disease of the newborn. Alloantibodies. Direct antiglobulin test. Monocyte monolayer assay.**

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Anti-U was first reported by Wiener et al.<sup>1</sup> in 1953. It was found in a black patient who had a fatal hemolytic transfusion reaction. The authors also reported the high frequency of U antigen on red cells; it was of near universal occurrence.

U antigen is part of the MNS blood group system. This complex system of over 40 antigens is carried on 2 glycoproteins molecules or hybrid molecules of the 2 proteins. Regarding transfusion medicine, M, N, S, s, and U antigens are the most important ones. The M and N antigens are located on glycoprotein A (GPA), and S, s, and U antigens are on glycoprotein B (GPB), encoded by GYPA and GYPB genes respectively. The GYPA and GYPB are members of the glycoprotein

gene family encompassing a 330-kb genomic segment located, in a very close proximity, on chromosome 4, band q31<sup>2</sup>. The GYPA gene consists of 7 exons and has 97% sequence homology with GYPB, which has 5 exons.

Glycophorins A (GPA) and B (GPB) are major sialoglycoproteins of the human erythrocyte membrane that bear the antigenic determinants for the MN and Ss blood groups. In addition to the M or N and S or s antigens that commonly occur in all populations,

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about 40 related variant phenotypes have been identified. These variants include all the variants of the Miltenberger complex and several isoforms of Sta, and also, Dantu, Sat, He, Mg, and deletion variants Ena, S-s-U-, and Mk. Most of the variants are resulted from gene recombinations between GYPA and GYPB<sup>3</sup>.

The high-incidence U antigen is a labile structure, located near the cell surface and is resistant to proteases<sup>4</sup>. U antigen appears to be well developed at birth. Marsh et al.<sup>5</sup> reported U antigen on neutrophils through adsorption studies.

Less than 1% of blacks are U-negative and capable of producing alloanti-U. In a study in 2,462 Brazilian blood donors, all whites and

browns were U-positive, and only 0.87% of blacks were U-negative. All of U-negative blood donors were also S-negative and s-negative simultaneously<sup>6</sup>.

Anti-U are generally non-complement binding IgG antibodies<sup>7</sup> containing an IgG 1 component<sup>8</sup>, are uncommon but are a recognized cause of hemolytic disease of the fetus and newborn (HDN).

There are 16 reports of HDN due to anti-U including 1 resulting in still-birth, all of them in black pregnant women.

In this study, we present the first case of HDN caused by anti-U identified in a brown woman.

## CASE REPORT

A female newborn delivered at 38 and 2/7 weeks, weighing 2,960 g, Apgar score 9/9, presented a strongly positive direct antiglobulin test with evidence of clinically significant mild hemolysis. Anti-U was recovered from an acid eluate of cord blood. Anti-UPR was identified as subspecificity due to its resistance to papain-treatment. The baby's red blood cells phenotyped as M+N+S-s+U+He-. The mother, a brown female, 36 years old, presented positive screening of antibodies (Indirect Coombs test) at delivery. An anti-U reacting at antiglobulin phase was identified. The antibody was of IgG1 and IgG3 subclasses and showed a titer of 32 by antiglobulin test. The red blood cells phenotyped as M+N-S-s-U-He-. Unfortunately it was not possible to obtain prenatal data of this pregnancy. She had had 4 earlier pregnancies. In the last pregnancy, 3 years ago, no irregular antibodies were detected in the serum. A monocyte monolayer assay<sup>9</sup> was devised to attempt to forecast *in vivo* red cells survival from *in vitro* test. The monocyte monolayer assay showed moderate interaction of Fc

receptors with maternal serum, with a positive result of 3.1%. Thirty-five hours after birth, bilirubin was 6.9 mg/dL, reticulocytes 2.4%, hemoglobin 12.1 g/dL, and hematocrit was 33.9%. The newborn was only treated with phototherapy for about 48 hours, recovered well and was discharged on the 4<sup>th</sup> day of life.

## DISCUSSION

HDN was a significant cause of fetal mortality and morbidity until the introduction of amniocentesis, intrauterine transfusion, controlled early delivery, and exchange transfusion in the management of severely alloimmunized women and their fetuses<sup>10</sup>. Typically, the serological diagnosis of HDN includes a positive direct antiglobulin test on the infant's red blood cells and the presence of an IgG red cell alloantibody in both maternal and cord sera.

The severity of HDN can be assessed with certainty only by measurement of fetal parameters. However, these invasive procedures may be risky for the fetus. Initially, immunohematological tests are performed on maternal serum to identify antibodies with a potential risk of HDN.

Although anti-D is the most common cause of hemolytic disease of newborn, it should be remembered that antibodies against other red blood cell antigens, such as S, s, K, Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup> can cause severe and even fatal HDN<sup>7</sup>.

Antibody concentration is inferred by indirect antiglobulin technique titration, or where possible, is quantified<sup>11</sup>. Titration is the most common method used in a routine laboratory to estimate the strength of an antibody.

Smith *et al.*<sup>12</sup> recommended that an anti-U titer of  $\geq 128$  or more at  $\geq 17$  weeks of gestation is an indication for assessment of hemolysis in the fetus.

However, the authors found anti-U titer of 4,000 in a pregnant woman at 38 weeks with no evidence of HDN. They hypothesized that the destruction of the antibody-coated red cells directed against the fetal mononuclear phagocytes could be inhibited by HLA-derived maternal alloantibodies<sup>13</sup>.

In the literature there are reports of 16 pregnancies complicated by anti-U, all of them in black-origin individuals. The severity of HDN varied from asymptomatic to fatal. The first case of HDN due anti-U was related by Alfonso and de Alvarez in 1961<sup>14</sup> with no evidence of clinically significant hemolysis. Seven other cases presented no clinical signs of HDN (1 related by Tuck & Studd<sup>15</sup>, 2 by Dopp & Isham<sup>16</sup>, and 4 cases by Smith *et al.*<sup>12</sup>).

Smith *et al.*<sup>12</sup> reported a newborn with anti-U, who needed a simple red blood cell transfusion. In 3 instances, exchange transfusion was performed to manage HDN to anti-U<sup>17,18,19</sup>.

Gottschall<sup>20</sup> presented a case in which exchange transfusion was performed, but anti-D was also present, and the contribution of the anti-U to the hemolysis was therefore uncertain.

There was 1 report of intrauterine death due to anti-U in 1964<sup>21</sup>. Intrauterine transfusion procedure was performed 3 times in 1 fetus, in which the anti-U titer was 1,000 at 20 weeks of gestation and arose to 16,000 at 32 weeks of gestation when a cesarean section was needed. During the first day of life, the newborn was given a platelet transfusion and 2 exchange transfusions. After that the baby was transfused 3 times in the neonatal period<sup>12</sup>.

A severe HDN caused by anti-U was diagnosed retrospectively, when a 3-week-old black infant presented severe hemolytic anemia and anti-U was identified in his mother serum<sup>22</sup>.

Smith *et al.*<sup>12</sup> recommended that an anti-U titer of  $\geq 128$  or more at  $\geq 17$  weeks of gestation is an indication for

assessment of hemolysis in the fetus.

Unfortunately, it was not possible for us to assess prenatal laboratory tests of the patient we reported. We performed a monocyte monolayer assay (MMA) to forecast the severity of HDN. This method, as well as other in vitro cellular assays, is recognized to have a good correlation in predicting disease severity, although a high MMA activity does not always indicate a severe HDN<sup>23,24</sup>. In this report, the weak positive result at MMA (3.1%) is in accordance with the anti-U titer observed in the maternal serum (32) and with mild signs of HDN.

The mother's red blood cell MNS

phenotyping showed a very rare pattern (M+N-S-s-U-). In order to explain this phenotyping result, or the mother's RBCs could lack GPB, or the GPB could be hybrid<sup>25</sup>. Consequently, this patient can produce other antibodies against any antigen carried on GPB.

Despite the fact that in this report the HDN was of mild type, it is noteworthy that, as far as we know, this is the first report of HDN due to anti-U identified in a brown woman. This finding is particularly relevant in countries like Brazil, where there is a high miscegenation population rate, and the brown group is one of the

most prevalent<sup>26</sup>.

In conclusion, based on the knowledge that anti-U should be suspected whenever an antibody to high frequency antigen is detected in a serum of previously pregnant or transfused black individuals<sup>27</sup>, we suggest that this recommendation be extended for the brown group.

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#### RESUMO

NOVARETTI MCZ e col. - Doença hemolítica do recém-nascido devido a anti-U. **Rev. Hosp. Clín. Fac. Méd. S. Paulo** 58(6):320-323, 2003.

Anti-U é um aloanticorpo eritrocitário raro detectado exclusivamente em negros, que pode causar doença hemolítica do recém-nascido e reações transfusionais hemolíticas.

Relatamos o caso de um recém-nascido, de sexo feminino, que apresentou teste de antiglobulina direto

fortemente positivo, dirigido a um anticorpo IgG em sangue de cordão umbilical. Anti-U foi identificado por técnica de eluição ácida. A mãe apresentava pesquisa de anticorpos irregulares positiva com anticorpo anti-U, de subclasses IgG1 e IgG3, título 32, identificado ao nascimento. O ensaio de monocamada de monócitos apresentou resultado positivo (3.1%), mostrando uma interação moderada de receptores Fc com soro materno. O recém-nascido foi tratado somente por fototerapia durante 48 horas para uma doença

hemolítica leve. A criança recuperou-se bem e teve alta médica no quarto dia de vida.

Concluímos que quando um anticorpo contra um antígeno eritrocitário de alta frequência for identificado em gestantes negras e pardas, anti-U deve ser investigado.

**DESCRITORES: Anti-U. Titulação de anticorpo. Doença hemolítica do recém-nascido. Aloanticorpos. Teste de antiglobulina direto. Ensaio de monocamada de monócitos.**

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