Routine Cord Blood Direct Coombs Test in Rh Negative Pregnancies- Can it be discontinued?
Dr. Karthik Ram Mohan¹*, Dr. Ashish Kumar Simalti², Dr. K Satyanand³
¹Sr Adv (Pediatrics) & Neonatologist, Command hospital (EC), Kolkata, India
²Pediatric Intensivist, Army Hospital research and referral, New Delhi, India
³Consultant (Medicine), Command Hospital (SC), Pune, India

Abstract: Hemolytic disease of the newborn (HDN) is caused by maternal antibodies formed against fetal antigens inherited from father. The Direct Coombs test (DCT) or direct antiglobulin test (DAT) is used as a tool for screening these allo-antibodies coating the RBCs and it is positive in various categories of immune hemolytic anemia. This study was conducted to assess the usefulness of cord blood DCT in Rh negative pregnancies in an Indian hospital scenario. This is a retrospective observational study of infants born to Rh negative mothers in south India in which cord blood investigations (Blood group and Direct Coombs test) were analysed. Of the total 1487 live born during the above period, 71 babies were at risk for Rh isoimmunisation. Of the 49 babies with Rh positive blood groups, DCT was positive in only one baby. Hence the 70 DCT tests did not influence management decision. This study suggests that routine cord blood DCT does not appear to be useful in Rh negative pregnancies with no antenatal evidence of isoimmunisation. Larger studies may be planned to provide more evidence as to whether to discontinue the practice of use of cord blood DCT in India.

Keywords: Cord Blood, Direct Coombs Test (DCT), direct antiglobulin test (DAT), Rh Negative pregnancy.

INTRODUCTION
Hemolytic disease of the newborn (HDN) is caused by maternal antibodies formed against fetal antigens inherited from father. ABO blood group incompatibility and Rh antigens (predominantly D, c, and E) are the most clinically significant of these antigens, followed by Kell antigens.

Relatively rare groups of antigens included are Kidd and MNS families. Patel et al. estimated incidence of Rh incompatibility among Indian population to be 1.37%[1]. Reason for presence of these antibodies in mother (maternal alloimmunization) is usually exposure during previous pregnancy or abortion. The introduction of routine postnatal prophylactic anti-D immunoglobulin for Rh D negative women in the 1970s has reduced incidence of HDN significantly [2]. Rarely, maternal autoimmune hemolytic anemia may also produce HDN by passive transfer of hemolytic antibodies.

The Direct Coombs test (DCT) or direct antiglobulin test (DAT) is used as a tool for screening these allo-antibodies coating the RBCs and it is positive in various categories of immune hemolytic anemia. In HDN a positive DCT results from transfer of IgG antibodies across the placenta. These antibodies are present in maternal blood and are directed against antigens present on neonatal RBCs. These antibodies are responsible for destruction of neonatal (or even fetal) RBCs and also reduce their life span. This antibody mediated hemolysis results in hyperbilirubinemia and anemia producing the clinical picture of HDN. Positive DCT is considered a major risk factor for HDN leading to the severe hyperbilirubinemia and potential for neurotoxicity [3]. Collecting cord blood samples for DCT has been part of standard protocol for all babies born to mothers with Rh negative blood group[4]. Current practice in most hospitals is to perform a DC Ton cord blood samples of all infants born to Rh-negative mothers. However this practice is now being questioned as recent studies suggest that a positive cord blood DCT is poorly predictive of subsequent hyperbilirubinemia[5].

The British Committee for Standards in Hematology guidelines no longer recommend that...
DCT be done on umbilical cord blood in all infants born to mothers with Rh D-negative blood group[6].

This study was conducted to assess the usefulness of cord blood DCT in Rh negative pregnancies in an Indian hospital scenario.

MATERIALS AND METHODS

This is a retrospective observational study of infants born to Rh negative mothers. Data of Rh negative pregnancies for the 18 month period of 01 Mar 2014 to 31 Aug 2015 of are ferral hospital in south India were analyzed. From the records, babies at risk (ie. born to Rh negative mothers with Rh positive fathers) were identified and cord blood investigations (Blood group and Direct Coombs test) were analysed.

**RESULTS**

Of the total 1487 live born during the above period, 71 babies were at risk for Rh isoimmunisation (Rh negative mother and Rh positive father). Antenatal ICT (Indirect Coombs test) was negative in all pregnancies at risk. 22 babies were detected to have negative blood group in cord blood investigations (Table 1). Of the 49 babies with Rh positive blood groups, DCT was positive in only one baby. That baby was closely monitored clinically and never developed icterus requiring either exchange transfusion or phototherapy. Hence the 70 DCT tests did not influence management decision. During the 18 month period, only one baby required exchange transfusion for Rh isoimmunisation and the baby was an out born baby born to an Rh negative mother with positive ICT test and whose cord blood (Done at referring hospital) revealed positive blood group with positive DCT report.

**Table-1: Data from Rh negative pregnancies (18 months from 01 Mar 2014 to 31 Aug 2015)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Live Deliveries</td>
<td>1487</td>
<td></td>
</tr>
<tr>
<td>Pregnancies at risk for Rh isoimmunisation*</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Positive ICT results</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Births to “At-risk” pregnancies</td>
<td>71</td>
<td>2 twin pregnancy.</td>
</tr>
<tr>
<td>Total Cord Blood samples</td>
<td>71</td>
<td>4.7% of all deliveries. DCT done in 70 cases.</td>
</tr>
<tr>
<td>Rh Negative Babies</td>
<td>22</td>
<td>DCT done in 21 cases. All negative</td>
</tr>
<tr>
<td>Rh Positive babies</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Positive DCT result</td>
<td>01</td>
<td>Baby did not require ET/PT</td>
</tr>
<tr>
<td>Negative DCT result</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Total DCT tests done</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>

(*Lady Rh negative and spouse Rh positive)(ET: Exchange Transfusion, PT: Phototherapy)

**DISCUSSION**

With advances of our understanding in the mechanism of HDN it was seen that most woman get exposed to Rh antigen during delivery or abortion if baby or abortus has Rh positive blood group. This sensitization can be prevented with prophylactic use of Anti D immunoglobulin for woman with Rh negative blood group and Rh positive fetus or baby within 48 hours of delivery or abortion. Use of Anti D is now routine and has led to significant reduction of alloimmunisation of Rh negative women thus reducing overall risk of HDN among this particular group. A positive DCT has always been one of the cornerstones of diagnosis of HDN.

Coomb’s test is a type of agglutination test done to detect antibodies (or complement) responsible for hemolysis by using anti-human globulin to produce an agglutination reaction. The indirect method detects antibodies which are circulating in the plasma, while the direct method tests for antibodies (or complement) which is bound to the RBC membrane, indicating pre-existing sensitization in vivo. Although first conceived by Moreschi in 1908, it was first used in clinical medicine by Robin Coombs in 1945[7]. Different reagents are available which can be either specific detecting either bound complement (C3) or bound immunoglobulin G (IgG) or nonspecific reagent which can simultaneously detect both antibody as well as complement. For screening purpose nonspecific reagent is used and if results are positive, specific reagents are used to distinguish between antibody and complement. Probability of a positive yield is higher among population considered to be at higher risk. Babies born to Rh negative mother is one such population if mother has been exposed to Rhesus antigen earlier and baby has Rh positive blood group. ABO incompatibility between mother and fetus is present in 15% to 25% pregnancies but only 1% produce positive DCT result on cord blood testing[8]. Out of these babies with DCT positive result, only 23% develops jaundice hence cord blood sampling for DCT is not recommended for ABO incompatibility [8, 9].

In this study, cord Blood DCT was performed in all babies born to Rh negative mothers (With Rh positive husbands). Importantly, all ladies had negative ICT results, possibly pointing to low rates of antenatal Rh isoimmunisation in the present era of extensive use of prophylactic anti-D immunoglobulin for Rh negative women. Out of the 70 DCT tests, 21 tests were unnecessary as baby’s blood group was Rh negative.
Even among 49 other tests where there was an incompatibility of Rh antigen, positive yield was very low with only 1 test coming as positive thus giving a positive yield of only 2% among high risk population. This yield is lower than reported by Valsami S et al. who found prevalence of DCT positive results to be2.59 % but they included all deliveries with bulk(91.3%) being ABO incompatibility[10]. Jude et al have suggested that routine use of cord blood for DCT testing is not to be recommended in mothers having Rh (D)-negative blood group because of large number of positive DCT may occur due to routine use of antenatal anti-Rh D prophylaxis[11]. This observation is at variance with our results. It could be because we used the classic tube method which has lower sensitivity as compared to newer gel/solid phase methods. Valsami S et al. found no false positive DCT attributable to anti-Rh D prophylaxis[10]. In our study even the baby who tested positive also did not develop significant hyperbilirubinemia, thus raising concerns regarding utility of this practice of collecting cord blood samples and performing DCT as a routine screening test for all babies born to mothers with Rh negative blood group. The incidence of a positive DCT in newborns ranges from 2.3-3.5 % among various studies [12, 13]. Among various studies positive predictive value of DCT has been variable in range of12%–53% and a sensitivity of 15%–64% for the subsequent development of hyperbilirubinemia [14].This limits its usefulness as a screening test. DCT fails to identify over half of the cases of significant hemolysis that are diagnosed by end-tidal carbon monoxide (the criterion standard for estimating the rate of hemolysis)[13]. Dinesh et al. reported that only 23% of neonates found to have a positive DCT on neonatal screening actually went on to develop hyperbilirubinemia requiring phototherapy. In that study, jaundice, rather than the positive DCT, was the first alert in the majority of cases of HDN requiring phototherapy [15]. Also, a study by Madan showed that routine DAT testing of cord blood from term non-jaundiced infants born to O positive mothers was not necessary[16].

There are also technical issues related to performance of this test. In the classical method, RBCs which are tested should be washed with saline in order to remove extra, free IgG or complement present in the serum which may inhibit the reagent and give false negative results. Another potential cause of false negative results is delay in performing the test after washing of RBCs cause bound antibody or complements may elute off. Newer automated methods are available which use gel phase or a solid phase and are easier to perform and have lesser chances of errors [17]. However, these methods have higher sensitivity as compared to conventional tube testing which may lead to detection of positive results of DCT which may not be relevant clinically and may add to unnecessary detailed evaluation and expenses [6, 18]. As mentioned earlier it has been suggested that, antenatal Rh Ig prophylaxis may lead to an increase in the positive DCT [11]. Similarly exposure to Methyldopa during pregnancy can also produce positive DCT. Carstairs et al. reported incidence of positive DCT post methyldopa exposure to be as high as 20% but only 2% babies subsequently developing hemolytic anemia [19]. Contamination of cord blood with Whartonjelly can also cause agglutination which is non specific and this can also cause false-positive DCT results [9]. ABO incompatibility also produces weak DCT positivity poorly correlating with subsequently clinical picture. Apart from this low predictive ability, the DCT is also costly when used as a screening test. US studies of the costs of evaluating neonatal jaundice have reported the cost per test to be US$17-$47[3]. Unexpected rapidly developing or severe hyperbilirubinaemia is now the clinical presentation that most often alerts clinicians to newborns with HDN. In a neonate with significant jaundice, a negative DCT result should prompt search for a cause other than is oimmunization even if there is incompatibility of blood group antigens between mother and the baby[20].

CONCLUSION
This study suggests that routine cord blood DCT does not appear to be useful in Rh negative pregnancies with no antenatal evidence of isoimmunisation. It should be reserved for cases where ICT is positive or there is significant HDN in a previous baby or in pregnancies where antenatal investigations were not done. Also, it is obviously unnecessary to perform a DCT when the baby is of Rh negative blood group. With standard use of anti D immunoglobulin and reduced number of pregnancies (Per mother), incidence of maternal alloimmunisation seems to have come down significantly. This has led to reduction in the prevalence of DCT positivity among neonates. With lower probability of positive tests, performing this test as a routine is no longer cost effective. What appears to be more important is close clinical observation for significant hyperbilirubinemia. Larger studies may be planned to provide more evidence as to whether to discontinue the practice of use of cord blood DCT in India.

REFERENCES