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ORIGINAL ARTICLE

Does strengths of a positive direct antiglobulin test predicts the need for phototherapy and duration of phototherapy? – a single center, retrospective study

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ABSTRACT

Background: Use of Direct Antiglobulin test (DAT) in management of neonatal hyperbilirubinemia is conflicting.

Objective: whether strength of positive DAT predicts the need for phototherapy, duration of phototherapy and need for major interventions.

Methods: We retrospectively collected data on all DAT positive neonates with birth gestational age ≥32 weeks over six years (2014–2019). Data regarding blood group, DAT and clinical details were obtained from a hospital database. We also collected data on serial hemoglobin and other relevant laboratory parameters. We also collected data on infants receiving major interventions such as exchange transfusion, in-utero transfusion, immunoglobulins, and postnatal transfusion for the duration of the study period. All of these infants were electronically followed up for a period of 6 weeks. This study was approved by institutional audit authority. All the statistics were performed using SPSS software.

Results: Out of 1285 DAT tests performed, only 91 infants were positive (7%), and 78 DAT positive infants were available for analysis. There were 54 infants with DAT (1+), 15 infants with DAT (2+), 7 infants with DAT (3+) and 2 infants with DAT (4+). There was no significant statistical difference in terms of need for phototherapy, duration of phototherapy, need for major interventions and hemoglobin levels at different time points between the groups (DAT 1+ Vs DAT ≥2+; DAT ≤2+ Vs DAT >2). A Total of 10 infants received major intervention, with one infant receiving all three interventions (DAT 3+ with significant maternal antibodies), 2 additional infants (both DAT1+) received exchange transfusion, 6 additional infants received immunoglobulin (2 infants: DAT 2+; 4 infants: DAT 1+) and one additional infant (DAT 1+) with significant maternal antibodies received a postnatal transfusion.

Conclusion: Strength of a DAT did not predict the need for phototherapy, duration of phototherapy, and the need for major hemolysis related intervention in the first 6 weeks of life.

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Direct antiglobulin test; hyperbilirubinemia; haemolysis; jaundice; phototherapy

Background

Direct Antiglobulin test (DAT) detects the presence of immunoglobulin or complement bound to red blood cell membrane. Suspected hemolytic disease of newborn (HDN) due to ABO incompatibility or Rhesus disease (Rh) is one of the most common indications for performing a DAT [1]. The study by Shahid et al. have reported the incidence of ABO-HDN only as 0.02–1.4% for all births [2]. Approximately 15% positive of DATs is due to the routine use of maternal anti-D immunoglobulin in Rh negative blood group mothers and passive transfer of immunoglobulins to the infants [3]. In some units it is a common practice to routinely perform cord blood DAT testing for maternal O/Rh Negative blood group [2].

There are some variations in the current clinical guidelines recommended by agencies [4,5]. National Institute for Health and Care Excellence (NICE) 2010 guidelines recommend performing DAT in infants with significant hyperbilirubinemia [4]. American Academy of Pediatrics recommends performing a DAT if the maternal antibody screen in positive or unknown [5].

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There is conflicting evidence suggesting usefulness of performing DAT in the management of neonatal hyperbilirubinemia [6–10]. We hypothesized that the higher strength of DAT would signify a greater proportion of immunoglobulins bound to RBC with higher chance of hemolysis and at a high risk for receiving phototherapy. The objective of this study is to find out whether the strength of positive DAT in infants born to mothers with any blood group predicts the need for phototherapy, duration of phototherapy and the need for major interventions for HDN such as exchange transfusion.

Methods

This retrospective study was conducted in a regional perinatal center over a period of six years (2014–2019). Data regarding blood groups and DAT performed in the neonatal unit and postnatal ward were obtained from a blood bank database. We included data for all DAT positive infants. Demographic and clinical data for DAT positive infants were obtained from a hospital electronic database and clinical notes. We followed up all DAT positive infants electronically for a period of 6 weeks as we expected all hemolysis related events would occur within this period. This is also to assess whether any of these DAT positive infants were readmitted due to hemolysis. Irrespective of their DAT status, we also collected details on infants receiving major intervention, defined as receiving postnatal blood transfusion for hemolytic anemia, immunoglobulins (IVIG), exchange transfusion and in-utero transfusion during this 6-year study period. This would provide us the total number of all the major interventions. We excluded preterm infants (<32 weeks) from our analysis as their jaundice could be primarily related to prematurity rather than hemolysis. It was routine practice in our center to perform a cord blood DAT, full blood count and serum bilirubin for infants at risk of HDN and significant hyperbilirubinemia in the first 24 h (See appendix-1 on our hospital guidelines). All DAT positive infants were clinically reviewed soon after birth and repeat full blood counts were performed at the age of 2 weeks and 6 weeks. In our center, we used a gel method for performing DAT and based on the strength of agglutination, DAT was graded from 1+ to 4+. We followed National Institute for Health and Care Excellence (NICE) guidelines and treatment threshold for management of jaundice [4]

We used a standard excel sheet for data collection. As this is a retrospective study of using data of all the DAT infants, we did not calculate sample size. We also used descriptive statistics for population characteristics. Categorical variables were presented as proportions, while numerical variables were presented as mean with a standard deviation or median with interquartile range as appropriate. We used Chi square/Fisher's exact test for categorical outcomes and independent t test/Mann Whitney U test for continuous outcomes based on the data distribution. All statistics were performed using IBM SPSS Statistics (version 23; IBM Corp., Armonk, New York). This study was registered and approved by the institutional audit authority. We used The Strengthening the Reporting of Observational Studies in Epidemiology statement (STROBE) guidelines for reporting of the study (Supplementary file) [11].

Results

Over a period of six years, there were 1285 DAT tests performed from the neonatal unit and postnatal ward. Only 91 infants were DAT positive, and we had a total of 78 infants available for the final analysis (Figure 1). In our study cohort, mean birth gestational age ± standard deviation (SD) was 37.8 ± 2.4 weeks and mean birth weight (SD) was 3068 ± 623 grams. There were 22 (28%) preterm infants with gestational age between 32 and 37 weeks. There were 39 (50%) male infants in the study. There were 54 infants with DAT (1+), 15 infants with DAT (2+), 7 infants with DAT (3+) and 2 infants with DAT (4+). Table 1 shows distribution of strength of DAT positive and neonatal blood groups.

Table 2 shows the clinical and laboratory characteristics for infants with DAT 1+ versus DAT≥ 2+. There was no statistical difference between the two groups with regards to receiving any major interventions, number of infants receiving phototherapy, duration of phototherapy and haemoglobulin levels at different time points. Comparison between infants with DAT ≤ 2 ($n = 69$) and DAT > 2 ($n = 9$), there is no significant difference in any of the parameters. Figure 2 shows the poor relationship between strengths of DAT and the highest bilirubin measured in each infant. In this graph we plotted all the individual infants' DAT positive results in the X-axis with their corresponding highest bilirubin level in the Y-axis.

One infant with (DAT 3+) in our study cohort received all three major interventions: antenatal transfusion, postnatal immunoglobulin, and exchange transfusion. In this case, maternal anti-Fya and anti-Jka was detected antenatally. Two other infants in our

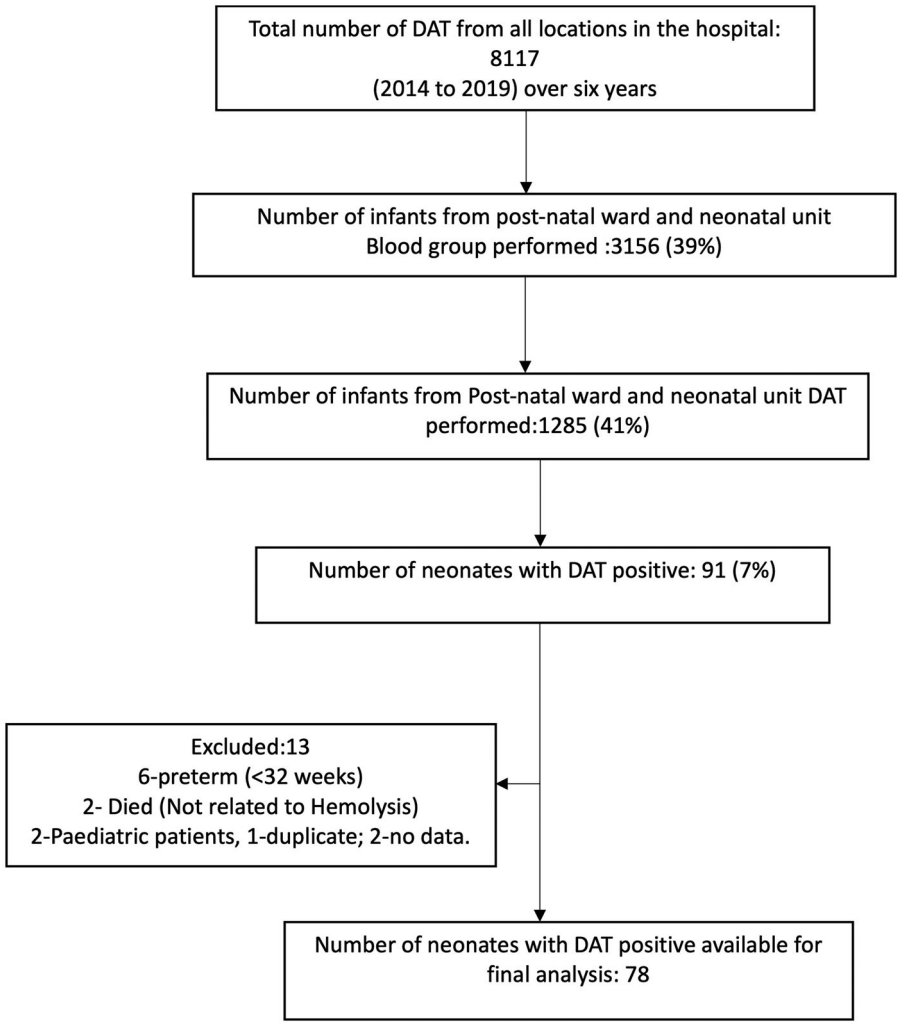

Figure 1. Study flow diagram.

Table 1. Distribution of DAT strengths by neonatal blood groups.

Neonatal blood group	DAT 1+	DAT 2+	DAT 3+	DAT 4+
O ($N = 23$)	16	4	2	1
A ($N = 44$)	30	9	4	1
B ($N = 9$)	7	2	0	0
AB ($N = 1$)	0	0	1	0
Not documented	1			

study received an exchange transfusion, with no maternal antibody detected. In both of these cases, very high bilirubin at admission seems to be the main reason for an exchange transfusion rather than any evidence of hemolysis. In both infant's O/A incompatibility was suspected with DAT 1+. We did not have details regarding clinical presentation in these infants. Seven infants received immunoglobulin, with two infants known to have significant maternal antibody titers (anti-Fya & anti- Jka; anti-E & anti-S antibodies). In the other 5 infants ABO incompatibility was suspected and there was no clear evidence of hemolysis. All of these infants received phototherapy and using immunoglobulin was under the discretion of the medical team. One infant (DAT 1+) with maternal e-antibody positive received a post-natal blood transfusion for hemoglobin of 68 g/dl at the age of 37 days.

In our study cohort, 11 infants were discharged on folic acid (DAT 1+: 7; DAT 2+: 3; DAT 3+: 1). There is no significant difference in hemoglobin and mean corpuscular volume at 2 weeks and 6 weeks in infants discharged on folic acid versus infants not discharged on folic acid ($n = 67$).

Discussion

From our study from high resource setting, we have shown that the strength of a positive DAT was not predictive for hyperbilirubinmeia needing phototherapy or the need for major interventions for hyperbilirubinemia and this is similar to the results of a few other studies [7,12,13]. On the contrary, few other studies reported good predictive value for DAT in the

Table 2. Clinical and laboratory characteristics for infants with DAT 1+ versus DAT≥ 2+.

	DAT 1+ $N = 54$	DAT ≥2 (DAT 2,3,4) ($N = 24$)	Relative Risk /mean difference (95% CI)	Statistics
Mothers with negative blood group	18/29 (62%)	7/14 (50%)	0.85 (0.54 to 1.3)	0.45
Number of infants received blood transfusion in the first 6 weeks	1/54 (1.85%)	0/24	0.73 (0.03 to 17)	0.85
Number of infants received Antenatal transfusion	0/54	1/24 (4.2%)	Not applicable	0.13
Number of infants received Exchange transfusion	2/54 (3.7%)	1/24 (4.2%)	0.96 (0.42 to 2.2)	0.92
Number of infants received Immunoglobulin	4/54 (7.4%)	3/24 (12.5%)	0.81 (0.42 to 1.6)	0.47
Number of infants received Phototherapy	33/54 (61%)	18/24 (75%)	1.2 (0.9 to 1.6)	0.23
Day of life phototherapy started; Median (IQR[b])	2 (1–3.25)	2 (1–2)	−0.81 (−1.6 to −0.05)	0.07
Number of days on phototherapy; Median (IQR)	1.75 (1–4)	2 (1–3)	−0.43 (−1.5 to 0.6)	0.94
Duration of hospital stay; Median (IQR)	4 (4–6)	4 (3–7)	0.50 (−2.3 to 3.3)	0.84
Haemoglobulin at birth in gm/dl; Mean ± SD[a]	167 ± 23	164 ± 25	−3.02 (−15 to 9.2)	0.62
Haemoglobulin at discharge in gm/dl; Mean ± SD	128 ± 32	123 ± 34	−5.5 (−22 to 10.8)	0.50
Haemoglobulin at around 2 weeks in gm/dl; Mean ± SD	143 ± 26	140 ± 27	−2.8 (−18.5 to 12.8)	0.72
Haemoglobulin at around 6 weeks in gm/dl; Mean ± SD	106 ± 13	107 ± 15	0.65 (−7.4 to 8.6)	0.87

[a]SD: Standard deviation; [b]IQR: Inter-quartile range.

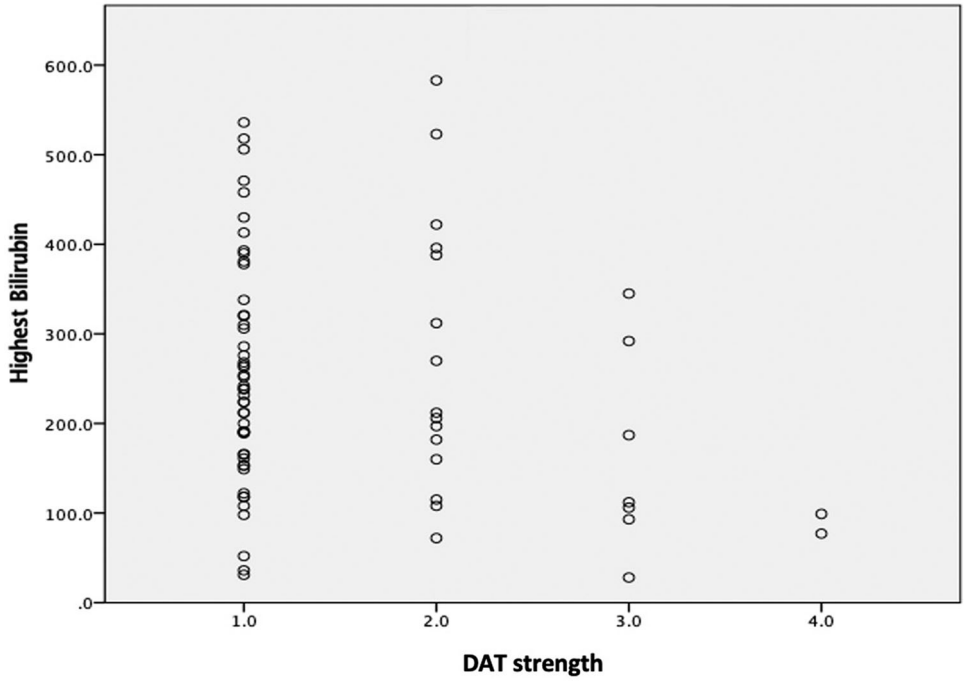

Figure 2. Relationships between grading of positive DAT and highest bilirubin levels. In this graph we plotted all the individual infants' DAT positive results in the X-axis with their corresponding highest bilirubin level in the Y-axis.

management of neonatal hyperbilirubinemia [6,8]. In a study correlating strength of DAT and hyperbilirubinemia, they used carbon mono-oxide (CO) as a marker for hemolysis [6]. This study included only DAT positive and ABO heterospecific blood group infants. In this study not all of the infants with DAT 2+ developed jaundice needing phototherapy (75%). Though, in this study no infant had more than DAT > 2+ they have concluded to use DAT strength rather than the presence or absence of DAT should be considered in the management of neonatal hyperbilirubinemia. In another study using end-tidal CO as a reference, sensitivity of DAT for prediction of hemolysis was only 38.5% [9]. For significant jaundice, end-tidal CO had a better positive predictive value than DAT (65.4% versus 52.9%).

Previous studies have reported positive DAT has poor positive predictive value of 12%-53% and sensitivity of 15%-64% for subsequent development of hyperbilirubinemia [13]. Negative DAT does not rule out ABO HDN and does not exclude non-immune hemolysis like G6PD deficiency and hereditary spherocytosis. Contamination of the cord blood with Wharton jelly and technical issues like the washing technique, centrifugation, reagent issues could cause both false-positive and false-negative DAT results [1]. Even with these small limitations, umbilical cord blood sampling should be the preferred method of blood

sampling in these situations, as it avoids venipuncture of the infants. There could be few reasons for the difference in results reported in these studies. The need for phototherapy treatment is based on different treatment thresholds; for example, American Academy uses risk-based bilirubin charts, whereas NICE uses only gestation specific bilirubin graphs. It could be due to the difference in ethnicity, level of liver conjugating enzyme in different populations, bilirubin excretion capacity and feeding practices.

Similar to our study, in clinical practice many infants with high bilirubin levels underwent DAT testing and receive major interventions like immunoglobulin without any evidence of immune hemolysis. In addition to the above laboratory tests, clinicians could use other markers of hemolysis (e.g. high reticulocyte count, raised serum lactate dehydrogenase enzyme levels, etc.) for decision making. This could be due to over reliance on DAT, physician perception that IVIG treatment is innocuous and the urge of physicians to avoid exchange transfusion. IVIG administration is not without any complications, its administration in itself could increase the risk of hemolysis as IVIG contains both anti-A and anti-B[1]. In an observational study over 16 years, the use of IVIG was strongly associated with developing necrotizing enterocolitis with an odds ratio of 31.6 (95% CI: 3.25–308.57)[14]. This was attributed to thrombotic effects caused by hyper viscosity of IVIG solution. In a systematic review, efficacy of IVIG was inconclusive for both Rh and ABO HDN with high risk of bias [15].

We could limit performing DAT tests, blood group and full blood count in high-risk cases like suspected immune hemolysis, jaundice needing treatment within 24 h, previous sibling receiving major interventions, signs of bilirubin encephalopathy, persistently rising bilirubin levels in spite of phototherapy, bilirubin close to exchange levels and prior to neonatal blood transfusion. If we follow this risk-based approach, in our center we could have reduced more than 1000 blood groups, full blood counts and DATs performed. Apart from cost savings of approximately 6000 GBP (Blood Group: £2.63/test; DAT: £1.52/test; FBC: £1.90/test)[13], it would avoid unnecessary blood sampling and parental anxiety. Moreover, limiting the practice of performing serial full blood count (at 2 and 6 weeks) in certain high-risk infants as mentioned above, would hugely reduce unnecessary blood sampling, parental travel time, hospital staff cost and parental anxiety. In a pre- and post-intervention study of introducing selective cord blood testing for mothers with O blood group, there was a cost saving of 4100 US dollars per year to the hospital and a reduction in 95 h of technician time to perform these tests [2]. There is no difference in jaundice levels and phototherapy treatment between the two periods.

Some centers have adopted testing of fetal blood group antigens in mothers with Rh Negative blood group. This practice negated the need for routine cord blood testing in Rh negative pregnancy. Similarly, few centers practice routine cord blood testing in mothers with blood group O. This practice has not shown to be cost effective if there is optimal surveillance strategy for hyperbilirubinemia, clinical risk assessment and with appropriate community follow up [16].

One of the major limitations in our study is that we did not collect data on infants with DAT negative compared with any DAT positive results. Another limitation of our study is our small sample size of DAT positive ($\geq$2+) infants, which is similar to previously published studies [6–8]. Our study had few strengths. We looked at all the clinically meaningful outcomes such as the need for phototherapy, duration of phototherapy and hospital stay. We looked at all infants receiving hemolysis related major interventions and followed up all of these infants up to 6 weeks of age. We also reported Haemoglobulin levels at various time points.

Hyperbilirubinemia alone should not be an indication to perform DAT [1]. DAT positivity or strength of positivity should not be the only criteria for making treatment recommendations for neonatal hyperbilirubinemia. Also, a positive DAT need not necessarily translate into severe HDN requiring interventions, but it could caution the caregiver to be more alert to the possibility of HDN. DAT should be used in conjunction with other laboratory test (e.g. Retic count) and clinical signs in suspected HDN even in the absence of maternal antibodies or blood group incompatibility [1]. In these cases, DAT would help in determining whether hemolysis is immune mediated or not. Performing major interventions like prescribing immunoglobulins, performing serial full blood count and follow up of these infants only based on DAT positivity, would cause an unnecessary burden to health economics and parents.

Conclusions

In our study, strength of positive DAT positive in a developed world setting was not predictive of need for phototherapy, duration of phototherapy and need for major interventions such as exchange transfusions.

Ethical approval

Obtained from Hospital authority.

Author contribution

JI was responsible for data collection, drafting initial manuscript, and approved the final manuscript. **RK** was responsible for interpretation of data, drafting initial manuscript, and approved the final manuscript. **CE** was responsible for concept, data provision, interpretation of data and approved the final manuscript. **PL** was responsible for concept, design, data collection, interpretation of data, data analysis, drafting initial manuscript, and approved the final manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Disclosure statement

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Data availability statement

Original data would be available upon request. Please email your request to: pkannanloganathan@nhs.net.

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