PERSPECTIVE

ABO hemolytic disease of the newborn: a need for clarity and consistency in diagnosis

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The diagnosis of ABO hemolytic disease of the newborn (ABO HDN) has been the subject of considerable debate and clinical confusion. Its use as an overarching default diagnosis for hyperbilirubinemia in all ABO incompatible neonates regardless of serological findings is problematic and lacks diagnostic precision. Data on hemolysis indexed by carbon monoxide (CO) levels in expired air (ETCOc) and blood (COHbc) support an essential role for a positive direct antiglobulin test (DAT) in making a more precise diagnosis of ABO HDN. A working definition that includes ABO incompatibility, significant neonatal hyperbilirubinemia, and a positive DAT is needed to gain clarity and consistency in the diagnosis of ABO HDN. Absent a positive DAT, the diagnosis of ABO HDN is suspect. Instead, a negative DAT in a severely hyperbilirubinemic ABO incompatible neonate should trigger an exhaustive search for an alternative cause, a search that may require the use of targeted gene panels.

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INTRODUCTION

Since its seminal description as *icterus neonatorum precox* [1], the diagnosis of ABO hemolytic disease of the newborn (ABO HDN) has been the subject of considerable debate and clinical confusion. Zuelzer and Cohen wrote more than 60 years ago, ABO HDN "is a remarkable entity which has not been and perhaps cannot be adequately defined on either clinical or serologic grounds" [2]. Many might still agree. However, failure to have a clear working definition of ABO HDN is problematic as exemplified by the common clinical use of ABO HDN as an overarching default diagnosis for hyperbilirubinemia in all ABO incompatible neonates regardless of serological findings, a practice also evident in the medical literature [3–5]. Such broad application lacks diagnostic precision, leads to the overdiagnosis of ABO HDN, and results in the misattribution of significant hyperbilirubinemia to ABO HDN when other conditions may be causative. More than a misdiagnosis, this practice represents a lost opportunity to correctly identify the etiology of the marked hyperbilirubinemia.

Some might argue distinguishing ABO HDN from other hyperbilirubinemia diagnoses is often clinically inconsequential. However, such distinction is important when choosing the operative treatment thresholds for hyperbilirubinemia [6], and in diagnosing neonates whose total serum bilirubin (TSB) levels are in the severe (≥20 mg/dL), extreme (≥25 mg/dL), or hazardous (≥30 mg/dL) range, or in those who develop acute bilirubin encephalopathy and kernicterus [7, 8]. These conditions warrant accurate identification of the cause of jaundice to ensure appropriate treatment, to gain a deeper understanding of the etiologic heterogeneity of marked hyperbilirubinemia, for perinatal counseling and management of future siblings, and because some identified disorders will require further evaluation and differentiated treatment in follow-up (e.g., hereditary spherocytosis) [9, 10]. Delays in diagnosis may also impact later outcomes and management. In one case series of 12 undiagnosed pediatric patients with hereditary spherocytosis, 10 presented with severe anemia requiring inpatient transfusion, all of whom had a prior history of neonatal jaundice with TSB levels of ≥15 mg/dL [11].

ABO HDN—Diagnostic Considerations

It is generally understood that ABO HDN is a hemolytic condition mediated by transplacentally acquired maternal anti-A or anti-B antibodies bound to heterospecific neonatal type A or B red blood cells that results in significant hyperbilirubinemia [12]. It follows that the diagnostic criteria for ABO HDN should include (i) ABO incompatibility between mother and neonate, (ii) clinically significant hyperbilirubinemia, and (iii) evidence of an antibody-dependent hemolytic process typically defined serologically and most convincingly by a positive direct antiglobulin test (DAT, direct Coombs' test) on neonatal red cells [12]. When these clinical and serologic findings are present, nearly all would agree they constitute a diagnosis of ABO HDN [12]. ABO HDN often occurs in first-born neonates [13].

What should we make, however, of the hyperbilirubinemic, DAT negative, ABO incompatible neonate? Is there evidence that significant hyperbilirubinemia in DAT negative ABO incompatible newborns represents ABO HDN? Can we clinically distinguish who, if any, among DAT negative hyperbilirubinemic ABO incompatible neonates has ABO HDN? These questions are addressed in this brief review.

ABO HDN—Serological Considerations

The confusion surrounding the diagnosis of ABO HDN centers largely on its serological features, specifically the role played by the DAT, the elution test, and the indirect antiglobulin test (IAT, indirect Coombs' test) in the neonate. It is therefore essential to understand and differentiate the diagnostic relevance of these

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three serologic tests to ABO HDN. In doing so it is important to keep separate the use of serologic tests as diagnostic criteria for ABO HDN in the already hyperbilirubinemic neonate from their usage as cord blood screening indices to predict subsequent hyperbilirubinemia risk. These two tasks are different and define subgroups that do not necessarily align. Yet these tasks are often conflated, creating confusion in the literature and clinical arena. For the purposes of this review, only serologic features as they relate to the diagnosis of ABO HDN in the hyperbilirubinemic neonate will be considered.

ABO HDN—The clinical significance of a positive DAT

The common clinically available DAT testing methodologies include the conventional tube test and the microcolumn gel card and solid phase testing assays [14]. Although the latter two platforms are more sensitive and increasingly employed by transfusion medicine services, manual tube testing still accounts for greater than half of neonatal DAT testing in the United States and Canada [15].

A DAT can demonstrate the presence of anti-erythrocyte immunoglobulin G (IgG) antibody bound to the surface of the red blood cell, a precondition for immune-mediated hemolysis [14, 16]. In point of fact, it is difficult to attribute an immune dependent process to a neonatal hemolytic condition when serologic evidence of red blood cell-bound IgG antibody is absent. Circulating antibody not bound to the red blood cell surface does not cause hemolysis in neonates. Accordingly, a positive DAT for IgG, while not diagnostic in itself, is nonetheless a diagnostic cornerstone of IgG immune-mediated hemolytic conditions and clinically helpful in distinguishing between antibody mediated and non-immune causes of hemolysis [12, 14, 16, 17].

Earlier studies reported that DAT(+) ABO incompatible neonates evidenced a greater degree of hemolysis than those with a negative DAT based on differences in hemoglobin, reticulocyte count and TSB [18, 19]. More recent studies of hemolysis indexed by carbon monoxide (CO) levels in blood (carboxyhemoglobin, COHbc) or expired air (end-tidal CO, ETCOc) corrected for ambient CO have considerably strengthened the diagnostic relevance of the DAT to ABO HDN [20, 21]. CO is produced in equimolar amounts with bilirubin when heme is catabolized to bilirubin [22, 23]. These studies report that DAT(+) ABO incompatible neonates often demonstrate elevated COHbc and ETCOc concentrations [20, 21] that exceed published thresholds for an increased hemolytic rate in neonates [24–27] even as they evidence a spectrum of hemolytic severity. Notably, COHbc and ETCOc concentrations in DAT(+) ABO incompatible neonates are significantly higher than their DAT(−) ABO incompatible and ABO compatible counterparts [20, 21, 28]. No statistically significant differences were observed in these CO measurements between DAT(−) ABO incompatible and ABO compatible neonates [20, 28]. Moreover, the intensity of hemolysis as indexed by elevated levels of ETCOc in DAT(+) neonates is greatest during the first 12–24 h of life, consistent with the characteristic pattern of early onset hyperbilirubinemia risk in ABO HDN [20].

In addition, the level of hemolysis in DAT(+) ABO incompatible neonates correlates directly with the risk of developing clinically significant hyperbilirubinemia [20, 21, 28]. DAT(+) ABO incompatible neonates with the highest COHbc levels evidenced the greatest hyperbilirubinemia risk whereas the DAT(+) ABO incompatible neonates with the lowest COHbc levels were at the lowest hyperbilirubinemia risk [21, 28, 29]. Not surprisingly, increasing DAT strength correlates with a higher incidence of hyperbilirubinemia and higher COHbc concentrations [28, 29].

Having said that, there is a subset of DAT(+) ABO incompatible neonates who are not hemolyzing by these CO criteria [20, 30] and who are at no apparent increased hyperbilirubinemia risk. It is no surprise that not all DAT(+) ABO incompatible neonates develop significant hyperbilirubinemia. A positive DAT as an index of immune-mediated hemolysis is only a proxy for hyperbilirubinemia risk when hemolysis is robust or at least sufficient to produce an imbalance between bilirubin production and hepatoenteric bilirubin clearance that favors hyperbilirubinemia development [23, 27, 31].

However, is it possible that a positive DAT may not fully encompass the breadth of clinically relevant immune-mediated hemolysis? It is asserted that low levels of red cell bound IgG below the DAT detection limit may still be sufficient to cause clinically significant hemolysis and resultant symptomatic hyperbilirubinemia [32, 33]. This assertion is separate from any technical issues with the DAT testing (e.g., removal of antibodies by washing during the DAT testing procedure). Are there data to address the possibility of immune-mediated hemolysis in DAT(−) ABO incompatible neonates?

ABO HDN—The DAT(-) ABO incompatible neonate

Using the conventional tube test and the microcolumn gel card and solid phase DAT testing assays [14], the detection limit of a positive DAT is variably estimated to be ~100–150 molecules of IgG bound per cell [34–36]. A negative DAT thereby reflects a low level of IgG sensitization [37]. Early studies of DAT(+) ABO incompatible hyperbilirubinemic (TSB ≥ 10 mg/dL) neonates estimated that the number of anti-A or anti-B molecules bound to the infants' red blood cells were typically greater than or, on occasion, just above (mean ± SD: 424 ± 224 molecules/cell; median: 198 molecules/cell; range 126–1320 molecules/cell) the DAT detection limit [34]. In contrast, DAT(-) ABO incompatible neonates evidenced red cell bound IgG levels within or below the DAT detection range (mean ± SD: 113 ± 17 molecules/cell; median: 117 molecules/cell; range 90–126 molecules/cell) [34]. These findings align with the higher maternal IgG antibody titers reported in DAT(+) as compared with DAT(-) ABO incompatible neonates [38].

Notably, there is a direct relationship between the amount of IgG antibody bound to the red blood cell and the rate of red cell clearance from the circulation [39, 40]; the fewer IgG molecules bound, the slower the rate of red cell clearance (i.e., less hemolysis) [39, 40]. As a consequence, one would predict that the level of hemolysis and hyperbilirubinemia risk in the DAT(−) ABO incompatible neonate would be lower than that of the DAT(+) ABO incompatible newborn. More specifically, if antibody-mediated hemolysis were to occur, it would likely be within the less severe end of the hemolytic spectrum and marked hyperbilirubinemia unlikely to develop. Are low IgG levels below the DAT detection limit in DAT(−) ABO incompatible neonates sufficient to cause significant hemolysis?

In fact, there is some overlap between the lower range of ETCOc and COHbc levels in DAT(+) ABO incompatible neonates and the upper limits of those measures in their DAT(−) ABO incompatible counterparts [20, 21, 28]. However, this overlap is most evident within the non-hyperbilirubinemic DAT(+) cohort and not the hyperbilirubinemic DAT(+) subgroup [21]. Hyperbilirubinemia was defined as a TSB > 95% on the Bhutani nomogram [41] in that report [21]. Notably, the ETCOc and COHbc levels in DAT(−) ABO incompatible neonates are often low, frequently comparable with ABO compatible newborns [20, 28] and often below those typically associated with overt hemolysis. Nevertheless, some suggest that detecting low level RBC bound IgG, below the limits of DAT detection, by using more sensitive elution testing might clarify who amongst DAT(−) ABO incompatible neonates with symptomatic hyperbilirubinemia may have ABO HDN [4, 32, 33].

Elution testing

An eluate is obtained by detaching antibodies from the neonatal red blood cell and assessing their reaction with donor erythrocytes to identify the specificity of the bound antibodies. Elution testing is commonly performed whenever a positive DAT is detected. The eluate is typically positive in the DAT(+)

neonate [42, 43]. However, the antibody concentration in the eluate is not commonly quantified.

There has been longstanding interest in the potential utility of elution testing in diagnosing ABO HDN, either in lieu of a DAT or in neonates with a negative DAT [4, 32, 33]. In principal, the eluate should be more sensitive than the DAT in detecting red cell bound IgG due to concentrating the surface bound IgG. On occasion, an eluate is reported as positive when the DAT is negative [4, 33]. Earlier studies suggested using a positive eluate as a diagnostic test for ABO HDN [32], particularly if quantified [33]. In fact, Voak and Bowley proposed that the "minimum criteria for the diagnosis of ABO HD[N] are the serological demonstration of incompatible anti-A/B antibodies in an eluate from the baby's red cell" accompanied by significant jaundice during the first few days following birth [32]. However, Alter et al. from the same era, reported eluate positivity that was just slightly more frequent than DAT positivity among ABO incompatible neonates [43], and that only DAT positive neonates developed hyperbilirubinemia that necessitated exchange transfusion [18].

Current experience suggests that eluate positivity among DAT(-) ABO incompatible neonates is less common [4, 42, 44] and associated significant hyperbilirubinemia risk infrequent [44]. In one recent large clinical investigation, none of 2,310 DAT(-) ABO incompatible neonates had a positive eluate, whereas 689 of 690 (99.9%) DAT(+) ABO incompatible neonates were eluate positive [42]. A much smaller contemporary study reported 22 positive eluates among 60 DAT (-) ABO incompatible neonates (37%), however, only 2 of these 22 (9%) evidenced clinically significant jaundice (no bilirubin levels were measured) [45]. In another small series of 27 hyperbilirubinemic (TSB > 12.0 mg/dL [205 µmol/L]) ABO incompatible neonates only 4 of the 27 (14%) were eluate positive and DAT(−), whereas 22 of the 27 (82%) were both DAT(+) and eluate positive [46].

It is worth noting that if very sensitive methods are used, virtually all DAT(−) ABO incompatible neonates have detectable red cell bound anti-A or anti-B antibodies calculated at between 8 and 85 IgG molecules/cell [47]. However, neonates with this low level of bound IgG had no clinical evidence of active hemolytic disease [47].

Exactly how frequently a positive eluate occurs in DAT(−) ABO incompatible neonates, and what it signifies in terms of hemolytic risk, is currently unclear as it is not common clinical practice to perform elution testing in newborns absent a positive DAT and this question has not been subject to recent extensive clinical research. Case reports of DAT(−), eluate positive ABO incompatible neonates with severe hyperbilirubinemia are currently rare [44, 48] and presumed to be cases of ABO HDN based largely on ancillary findings e.g., early hyperbilirubinemia presentation coupled with high maternal anti-A or anti-B titers [44, 48]. The current paucity of such reports coupled with limited clinical data on the subject suggest that caution is warranted in diagnosing ABO HDN in DAT(−), eluate positive neonates with severe hyperbilirubinemia and only as a diagnosis of exclusion following an exhaustive search for another icterogenic cause [12, 20, 49, 50]. At the same time it does seem reasonable to conclude that hyperbilirubinemic DAT(−) neonates who are also eluate negative do not have ABO HDN.

Indirect antiglobulin test

A positive IAT in the neonate demonstrates the presence of free unbound anti-erythrocyte antibodies circulating in the serum. Although there was early interest in the potential diagnostic relevance of a positive IAT to ABO HDN [2, 13] including its use as an inclusion criterion in ABO HDN research [51], it is now widely recognized that a positive IAT is not presumptive evidence that red cells have bound IgG or are damaged by the circulating antibody [19]. Notably, in one of the largest studies of ABO HDN that assessed the IAT, ABO incompatible neonates with a positive IAT but negative DAT demonstrated cord hematocrits and a prevalence of peak serum bilirubin levels ≥12.8 mg/dL (219 µmol/L) (9.4%) that were not significantly different than their ABO incompatible counterparts who were both IAT and DAT negative (7.8%) [19]. In contrast, those ABO incompatible neonates with a positive DAT evidenced significantly lower cord hematocrits and a more than 2-fold higher prevalence (20.4%) of peak serum bilirubin levels ≥12.8 mg/dL (219 µmol/L) [19]. These findings led the authors to conclude the "presence of a positive ICT [IAT] result [in the neonate] in the face of a negative DAT result probably has epidemiologic significance only, conveys no useful clinical information, and thus has no role in the evaluation of jaundiced newborn infants" [19]. There is nothing in the current literature to suggest otherwise.

ABO HDN and Gilbert syndrome

Marked hyperbilirubinemia is unlikely to develop in ABO incompatible neonates if there is little to no hemolysis and/or hepatoenteric bilirubin clearance is robust [23, 27, 31]. But what if there is an element of hemolysis *and* impaired hepatic bilirubin clearance? The possibility that the reduced bilirubin conjugating capacity in Gilbert syndrome coupled with low grade hemolysis might combine to produce an ABO HDN phenotype in DAT(-) ABO incompatible neonates has been suggested by Kaplan et al. [52] and merits examination.

Gilbert syndrome is a common congenital inborn error of hepatic bilirubin conjugation wherein uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) isoenzyme activity is reduced by ~70% or more [53–55]. In a preliminary study of 40 DAT(−) ABO incompatible neonates, Kaplan et al. reported a strong positive association between their hyperbilirubinemia risk and the expression of the *UGT1A1* promoter variant *UGT1A1*28* [52], one of the polymorphic gene variants that underlies Gilbert syndrome [53–55]. More specifically, all DAT(−) ABO incompatible neonates with a TSB ≥ 15 mg/dL [256 µmol/L]) were either homozygous or heterozygous for *UGT1A1*28*. In contrast, none of the DAT(-) ABO incompatible neonates who were homozygous for the wild type allele (*UGT1A1*1*) evidenced a TSB ≥ 15 mg/dL [256 µmol/L]) [52].

Based on the reported *UGT1A1*28* allele frequency (0.35) in the Kaplan et al. paper and assuming a Hardy-Weinberg equilibrium, 12.5% of the entire study cohort of DAT(−) ABO incompatible were homozygous for *UGT1A1*28* and had Gilbert syndrome. Specifically, of the 40 DAT(−) ABO incompatible neonates studied, 5 were homozygous for *UGT1A1*28* and had Gilbert syndrome (of whom 2 were hyperbilirubinemic); 14 were heterozygous for *UGT1A1*28* (of whom 2 were hyperbilirubinemic) and 21 were homozygous for the wild type allele (none of whom were hyperbilirubinemic) [52].

These findings suggest that expression of the *UGT1A1*28* variant allele contributes to the development of hyperbilirubinemia in some DAT(−) ABO incompatible neonates. However, most studies report that the expression of the *UGT1A1*28* variant itself poses limited to no enhanced hyperbilirubinemia risk [52, 54–58]. Only when co-expressed with icterogenic conditions, such as breastfeeding or hemolytic disease, does *UGT1A1*28* augment the risk to develop significant hyperbilirubinemia [54, 59]. Thus, the Kaplan et al. findings infer that some, but not all, DAT(-) ABO incompatible neonates experience a degree of hemolysis sufficient in combination with impaired bilirubin conjugation to pose a hyperbilirubinemia risk. However, this level of hemolysis is insufficient *alone* to be icterogenic and, is therefore, presumed to be limited.

The preliminary findings of Kaplan et al merit confirmation. In this regard, Halis et al report an icterogenic effect of *UGT1A1*28* variant expression in DAT(−) ABO incompatible neonates but observed a similar association among ABO compatible newborns with unexplained hyperbilirubinemia as well [60]. These authors further suggest the *UGT1A1*6* variant has an icterogenic effect in

both DAT(−) ABO incompatible and ABO compatible neonates although the *UGT1A1*6* allele frequency in their study cohort was too low to draw conclusions [60]. *UGT1A1*6* is a missense *UGT1A1* coding sequence variant associated with a greater reduction in UGT1A1 conjugating activity than that seen in *UGT1A1*28* [54] and commonly underlies the Gilbert phenotype in East Asian populations [54, 55]. Yu et al have demonstrated that *UGT1A1*6* exerts a clear icterogenic effect in DAT(+) ABO incompatible neonates and/or those with laboratory evidence of hemolysis (reticulocytosis and spherocytes on peripheral smear) [61]. Notably this impact was most evident for TSB levels in the severe (>20 mg/dL [342 µmol/L]) and extreme (>25 mg/dL [>427 µmol/L]) range [61]. These findings are consistent with the Gilbert syndrome hyperbilirubinemia augmenting effect reported in hemolytic conditions [54, 55, 59].

Although polymorphic *UGT1A1* gene variants are common and can enhance hyperbilirubinemia risk, they are unlikely to account for all significant hyperbilirubinemia among DAT(−) ABO incompatible neonates. Clarification of the association between Gilbert syndrome and ABO HDN will require further clinical study using next generation sequencing panels that encompass variants of *UGT1A1* and other genes that underlie hemolytic conditions (see below).

Ancillary clinical findings

It is often asserted that ancillary clinical findings may assist in making the diagnosis of ABO HDN including a rapid early postnatal rate of rise in the serum bilirubin concentration, the presence of anemia and reticulocytosis, an elevated lactate dehydrogenase and low haptoglobin, and findings of microspherocytosis on peripheral blood smear. Indeed, one of the classic clinical features of symptomatic ABO HDN is its early temporal onset with a rapid rate of rise in TSB during the first 12 to 24 h of life. Hyperbilirubinemia presenting beyond 48 h of age is less often encountered and should always raise alternative diagnostic considerations [21]. However, since neonatal hemolytic conditions in general often present with clinical jaundice in the first 24 h of life, early onset jaundice is not unique to ABO HDN.

Similarly, reticulocytosis and an elevated lactate dehydrogenase when present lend credence to the diagnosis of hemolysis but are not specific to ABO HDN. Notably, anemia is distinctly uncommon in ABO HDN [13, 17] and ahaptoglobinemia is often found in normal neonates. Microspherocytosis on peripheral blood smear is also described in ABO HDN but hereditary spherocytosis can present a similar picture. In fact, cases of extreme hyperbilirubinemia in neonates with both DAT(+) ABO HDN and hereditary spherocytosis have been described [62]. As highlighted earlier in this review, measurement of end-tidal carbon monoxide production, corrected for ambient carbon monoxide, is increasingly available in the clinical arena and a useful method for quantifying hemolysis but again not specific to ABO HDN.

In contrast, elevated maternal anti-A and anti-B antibody titers are more specific to ABO HDN and often cited as supportive evidence in the diagnosis [44]. Not surprisingly, when assessed, maternal antibody titers are often elevated in symptomatic ABO HDN and are notably higher in mothers of infants with a positive DAT (geometric mean: 660 [95% CI: 414–1053]) as contrasted to their DAT(−) counterparts (geometric mean: 135 [95% CI: 988–208]) [34, 38]. Markedly elevated maternal titers (≥1:1024) are also reported in neonates with ABO HDN who required exchange transfusion [38, 63]. However, it appears that maternal titers in this elevated range (≥1:1024) are infrequent in DAT(−) neonates [34, 38] and others have found maternal IgG titers less informative than the DAT [42, 64].

Summary

The diagnosis of ABO HDN as the cause of significant hyperbilirubinemia requires more than the demonstration of blood group differences between mother and neonate [65]. As highlighted in this review, data on ETCOc and COHbc support an essential role for a positive DAT in making a more precise diagnosis of ABO HDN, a stance echoed by many [12, 19, 20, 49]. Adhering to a working definition that includes ABO incompatibility, significant neonatal hyperbilirubinemia, *and* a positive DAT is needed to gain clarity and consistency in the diagnosis of ABO HDN. Absent a positive DAT, the diagnosis of ABO HDN is suspect [20, 49]. Accordingly, the use of ABO HDN as a default diagnosis for all severely hyperbilirubinemic DAT(−) ABO incompatible neonates should be discouraged. Instead, a negative DAT in a severely hyperbilirubinemic ABO incompatible neonate should trigger an exhaustive search for an alternative cause [12, 20, 49].

This search strategy may require the use of next generation DNA sequencing (NGS) panels that target hereditary hemolytic anemia and genes involved in bilirubin metabolism [10, 62, 66, 67]. These panels include frequently encountered non-immune mediated hemolytic conditions (e.g., red cell membrane defects and enzymopathies) as well as *UGT1A1* gene variants that underlie Gilbert syndrome [10, 62, 66, 67]. Moreover, they have the capacity to identify novel pathogenic variants in these genes.

Others suggest using more sensitive DAT methodologies including flow cytometric IgG testing if conventional DAT results are negative [68]. However, it is increasingly clear that many DAT(−) ABO incompatible neonates with significant hyperbilirubinemia have an identifiable non-immune mediated hemolytic condition underlying their jaundice [20, 69]. Herschel et al. highlighted this phenomenon in a series of hyperbilirubinemic DAT(−) ABO incompatible neonates who were hemolyzing based on ETCOc criteria, all of whom had an identifiable non-immune mediated hemolytic condition (G6PD deficiency, elliptocytosis, G6PD deficiency coupled with Gilbert syndrome) [20]. Similarly, Christensen et al reported two cases of DAT(−) ABO incompatible neonates with extreme hyperbilirubinemia and acute bilirubin encephalopathy in a large cohort of >400,000 live births [50, 69]. Non-immune mediated hemolytic conditions were identified in both cases (hereditary spherocytosis and G6PD deficiency).

These studies highlight that rigorous efforts to improve the accuracy of an ABO HDN diagnosis will enhance our understanding of the broad nature of hemolytic conditions in the newborn and the full complexity of neonatal hyperbilirubinemia. Given the diagnostic tools now available to clinicians, failure to clearly identify the operative icterogenic condition(s) in cases of severe hyperbilirubinemia should be infrequent [69–71].

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The author declares no competing interests.

ADDITIONAL INFORMATION

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