Clinical value of combination detection of direct antiglobulin test and serum albumin globulin ratio in severe hyperbilirubinemia caused by ABO hemolytic disease of the newborn: A single-center retrospective analysis

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Research Article

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Abstract

Purpose To analyze clinical application of combination detection of direct antiglobulin test(DAT) and albumin globulin ratio(AGR) in severe hyperbilirubinemia caused by ABO hemolytic disease of the newborn(ABO-HDN).

Methods The measurement of DAT, AGR and combination detection of DAT and AGR was done to predict severe ABO-HDN hyperbilirubinemia in 270 full-term infants based on whether the infants received transfusions of blood components. The infants were divided into three groups according to the results of DAT and ARG and compared the differences of phototherapy day and hospitalization day of the three groups.

Results Of 270 cases enrolled in this study, 69 infants were DAT positive. ROC curve analysis showed that DAT cutoff value > ± with a sensitivity of 39.4% and a specificity of 83.9% and AGR cutoff value < 2.05 with a sensitivity of 54.1% and a specificity of 85.7%, the AUCs for DAT, AGR and combination detection of DAT and AGR were 0.621, 0.740 and 0.750 respectively. The phototherapy day and hospitalization day were significantly longer in group of AGR < 2.05 and DAT > ±than that of group of AGR < 2.05 and group of DAT > ±.

Conclusions DAT and ARG could be early predictors for the severity ABO-HDN hyperbilirubinemia and combination detection of DAT and AGR could further increase its predictive value.

Introduction

The pathogeny of hemolytic disease of the newborn (HDN) is maternal alloimmunization, the active IgG maternal red cell alloantibodies stimulated by fetal erythrocyte antigen could transport across the placenta and destroy involved fetal erythrocyte carrying the antigen[1]. HND is an important cause of neonatal morbidity and mortality with clinical presentation including mild clinical symptoms of neonatal jaundice, neonatal anemia and extramedullary hematopoiesis and severe symptoms of critical hyperbilirubinemia, anemia, hydrops fetalis even neonatal death[2]. ABO-HDN refers to occur mostly in infants with blood group A or B born to mothers with blood group O as the result of higher frequent occurrence of IgG anti-A and anti-B more in group O individuals, previous meta-analysis concerning the incidence of ABO incompatibility in pregnancies showed that about 15–25% of pregnancies can have ABO incompatibility and 10% develop HDN[3]. ABO-HDN has emerged as the leading cause of HDN and tends to be a milder disease usually treated successfully with phototherapy alone and rarely requires exchange transfusion therapy, intravenous immunoglobulin (IVIG) administration, or red blood cell (RBC) transfusion, compared with Rh-HDN, however, in severe cases, ABO-HDN may lead to severe fetal anemia with a risk for fetal death and to severe forms of neonatal hyperbilirubinemia with a risk for kernicterus or chronic bilirubin encephalopathy, which can lead to permanent brain damage[4], previous reports[5,6] have described the cases with severe anemia treated with RBC transfusion and with significant hyperbilirubinemia where exchange transfusion therapy is required. In our latest research[7] on exchange

transfusion therapy enrolled 123 infants with severe hyperbilirubinemia in Wuhan demonstrated that ABO-HDN is the leading etiology of severe neonatal hyperbilirubinemia which should be treated with exchange transfusion therapy. Delay in recognizing severe ABO-HDN may potentially leads to significant hyperbilirubinemia that may require intervention, therefore, early and rapid identification of severe ABO-HDN is critical for pediatricians to discriminate high risk neonates, enhance early recognition of potentially severe neonatal jaundice, decrease bilirubin levels and instate treatments to minimize further hemolysis, ameliorate anemia[8,9].

Currently, postpartum examinations of screening for ABO, Rh blood group type, irregular antibodies and direct antiglobulin test (DAT) are performed to identify neonates who are at higher risk for HDN in many hospitals[10]. DAT (previously termed direct Coombs' test), firstly described in 1945 by Cambridge immunologist Robin Coombs[11], has been widely used to detect antibodies or complement bound directly to the patient's RBCs, indicating in vivo sensitization to diagnose autoimmune hemolytic anemias as well as HDN, hence, DAT is regarded as the cornerstone in the diagnosis of HDN[12] and in 2016, the guideline of evidence-based recommendations for the application of blood grouping and red cell antibody testing in pregnancy to predict and prevent HDN developed in accordance with the British Committee for Standards in Hematology methodology showed that DAT on a neonatal specimen may help to determine the etiology of neonatal hyperbilirubinemia when HDN is suspected[13], however, the incidence of positive DAT in newborns with ABO-HDN is low, leading to the positive DAT or negative DAT may not rule in or out ABO-HDN. Additionally, the negative DAT may not exclude clinically significant hyperbilirubinemia caused by a non-immune hemolytic etiology including G6PD deficiency, sepsis, intracranial hemorrhage, hereditary spherocytosis and pyruvate kinase deficiency as well. Moreover, the low concentration of the anti-A or anti-B antibody occurs in ABO-HDN may not be detected by DAT because of the insufficient amount of anti-A or anti-B antibody, could still increase bilirubin level and generate mild hemolysis[14], meanwhile, the DAT itself may be affected by many factors including anti-RBC IgA, low affinity antibody, antihuman globulin activity and centrifugation technique, in our previous research which was concerning the interference in DAT showed that rheumatoid factor in under-tested samples can lead to both false decreases and false increases in DAT[15]. Therefore, DAT could not support the diagnosis of severe hemolysis alone and combined additional identified factors and investigations are required to diagnose the severity of ABO-HDN. Albumin globulin ratio(AGR), calculated as albumin/globulin, is actually a reflection of all non-albumin proteins, has been reported to be a novel prognosticator of many diseases including different types of cancer and some other diseases such as polyangiitis and heart failure[16,17]. The potential decrease of albumin concentration caused by bilirubin metabolism may lead to the decline of AGR level and no study has investigated the clinical value of association of AGR with the severity of ABO-HDN so far, therefore, in the study, our aim is to explore whether the combination detection of AGR and DAT would predict the severity of ABO-HDN.

Materials & Methods

Study subjects

From June 2020-December 2021, all the full-term suspected maternal-infant ABO blood group incompatibility infants in Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology were selected to perform elution test. The infants with positive elution test were identified as ABO incompatibility and enrolled in our research finally. Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology is the leading unit of the Hubei Pediatric Medical Union and Transshipment Center for critically ill children in Hubei, located in central China cited in Wuhan City, the capital of Hubei Province, located in the central part of the mainland China, received nearly 10,000 neonates from Hubei and surrounding provinces, including Henan, Jiangxi, Anhui and Hunan during 2020 to 2021. Exclusion criteria: (1) without hyperbilirubinemia, (2) gestational age less than 37 weeks, (3) with other causes may lead to hyperbilirubinemia, including sepsis, intracranial hemorrhage, G6PD deficiency, et al, (4) discharge required by guardians of neonates who did not meet the clinical cure standard, (5) received operations during hospitalization, (6) with history for treatment in other hospitals, (7) with severe congenital malformation.

Data collection

The basic conditions of all infants were collected from electronic medical record system anonymously, including age of hospitalization, sex, gestational age, delivery mode, feeding mode, age of jaundice, age of peak total bilirubin, birth weight, body weight at hospitalization, days of phototherapy, days of hospitalization, as well as the results of laboratory tests during hospitalization, including ABO blood type, peak total bilirubin, HGB, RBC, AGR and DAT at the same time, the general data of the mothers were recorded, including age, gestational day, pregnancy and delivery history and so on.

Outcome measures

The primary outcome was the infants received transfusions of blood components, including IVIG, albumin, RBC and exchange transfusion therapy during hospitalization. The secondary outcomes included the days of phototherapy and the length of hospitalization.

Statistical analysis

The measurement data were tested by Kolmogorov-Smirnov test or Shapiro-Wilk test to see if they were of normal distribution. Normally distributed data were expressed by mean ± standard deviation ($x \pm s$) and data that were not normally distributed were presented as median and 25th–75th centiles. A univariate binary logistic regression analysis was used to compare the differences of variables between the infants with and without blood components transfusion, after identification of predictors, a multivariate binary logistic regression analysis was performed to identify whether the AGR and DAT were ultimate predictors. A receiver operating characteristic (ROC) curve was made to compare the difference

of prediction value of AGR, DAT and combination detection of AGR and DAT and the result is presented as the area under the curve (AUC). To find the cutoff value for AGR and DAT as predictors for blood components transfusion in ABO-HDN infants, the Youden index was calculated. The Youden index is presented as the fetal AGR and DAT levels with corresponding sensitivity and specificity. A three-independent samples nonparametric test was performed to compared differences of phototherapy day and hospitalization day among

group of AGR < 2.05, group of DAT > ± and group of AGR < 2.05 and DAT > ±. The difference of P value less than 0.05 was considered to be significant. These analyses were performed using the SPSS 20.0 statistical software (IBM, Chicago, Illinois, USA). The figures were performed using the GraphPad Prism 8.0 software (San Diego, California, USA).

Results

During the study period, 510 infants were confirmed as ABO incompatibility and admitted to Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology, of which 465 cases were diagnosed with hyperbilirubinemia during the study period and thus were eligible for this study. A total of 185 cases were excluded, as 46 cases were discharge required by guardians of neonates, 43 cases were excluded because of the gestational age was less than 37 weeks, 52 cases with other causes of hyperbilirubinemia, including 40 cases of sepsis and 12 cases of intracranial hemorrhage, respectively, 21cases received operations during hospitalization, 19 infants has the history for treatment in other hospitals and 4 infants with severe congenital malformation. The derivation of the study population is shown in Fig. 1. The baseline characteristics of the study group are presented in Table 1. Finally, 270 cases were enrolled in this study, of which 109 infants received blood components infusion. Among them, 80 infants received one kind of blood components, 29 infants received more than one kind of blood components, respectively and the number of infants who received one time, two times, three times or more than three times of blood components infusions were 65, 29, 13 and 2, respectively.

Univariate binary logistic regression analysis of potential predictors for blood components transfusion was performed (Table 2). Peak total bilirubin (Crude OR, 0.991; 95% CI, 0.987–0.994), AGR (Crude OR, 2.819; 95% CI, 1.917–4.145), DAT (1+) (Crude OR, 0.356; 95% CI, 0.175–0.722), DAT (2+) (Crude OR, 0.201; 95% CI, 0.080–0.509), age of jaundice (Crude OR,1.208; 95% CI, 1.026–1.422), age of hospitalization (Crude OR,1.096; 95% CI, 1.011–1.188), birth weight (Crude OR, 0.999; 95% CI, 0.999-1.000), HGB (Crude OR, 1.021; 95% CI, 1.010–1.032) and RBC (Crude OR, 2.379; 95% CI, 1.619–3.496) were statistical significantly associated with need for blood components transfusion. The factors were entered in a multivariate logistic regression model to assess the independent association of these risk factors on the need for blood components transfusion (Table 2), peak total bilirubin (Adjusted OR, 0.970; 95% CI, 0.962–0.987), AGR (Adjusted OR, 6.152; 95% CI, 3.593–10.535), DAT (±) (Adjusted OR, 0.106; 95% CI, 0.018–0.646), DAT (1+) (Adjusted OR, 0.280; 95% CI, 0.116–0.674), DAT (2+) (Adjusted OR, 0.152; 95% CI, 0.049–0.472) were still independently associated with the need for blood components transfusion.

An ROC curve was plotted and the AUCs for DAT and AGR were 0.621 and 0.740, respectively. The Youden index was calculated at 0.398 with a cutoff AGR of < 2.05 with a sensitivity of 54.1% and a specificity of 85.7%, the Youden index was calculated at 0.233 with a cutoff DAT level of > ± with a sensitivity of 39.4% and a specificity of 83.9%. An ROC curve was plotted and the AUC for combination detection of DAT and ARG was 0.750 (Fig. 2).

A three-independent samples nonparametric test was performed and there are significant statistical difference of phototherapy day and hospitalization day among group of AGR < 2.05, group of DAT > ± and group of AGR < 2.05 and DAT > ± (Fig. 3).

Table 1
Baseline characteristics of study population

Characteristics	
Gender	
Male	147
Female	123
Delivery Mode	
Cesarean Section	115
Vaginal Delivery	155
Pregnancy and delivery history	
Yes	164
No	105
Feeding Mode	
Breast Feeding	37
Artificial Feeding	100
Mixed Feeding	133
Neonatal ABO Blood Type	
A	140
B	129
AB	1
Age of Mothers(y)	30.54 ± 3.78
Gestational Day(d)	273.41 ± 6.74
Birth Weight(g)	3329.46 ± 411.86
Hospitalization Weight(g)	3241.33 ± 421.03
Age of Jaundice(d)	1.00(0.00,2.00)
Age of Peak Total Bilirubin(d)	3.00(2.00,6.00)
Age of Hospitalization(d)	3.00(1.75,5.00)
Phototherapy Day(d)	6.44 ± 1.80
Hospitalization Day(d)	7.10 ± 2.03

Characteristics	
Peak Total Bilirubin(μmol/L)	256.85 ± 79.86
HGB(g/L)[a]	158.15 ± 24.15
RBC(10^{12}/L)[a]	4.58 ± 2.16
AGR[b]	2.51 ± 0.91
DAT	
Negative	201
Positive	69
±	7
1+	38
2+	23
3+	1
Blood Components	
No	161
Yes	109
Types of Blood Components Received	
IVIG	78
Albumin	1
Exchange transfusion therapy	1
IVIG + Exchange transfusion therapy	11
IVIG + RBC	8
IVIG + Albumin	8
IVIG + Albumin + Exchange transfusion therapy	1
IVIG + Albumin + RBC	1
Number of Blood Components Received	
One	65
Two	29
Three	13

Characteristics	
More Than Three	2
[a] From the first blood routine samples collected after admission	
[b] From the samples with the peak total bilirubin	

Table 2
Predictors for blood components transfusion in infants with ABO-HDN hyperbilirubinemia

Characteristic	P	Crude OR	95%CI		P	Adjusted OR	95%CI	
Peak Total Bilirubin	0.000	0.991	0.987	0.994	0.000	0.970	0.962	0.978
AGR	0.000	2.819	1.917	4.145	0.000	6.152	3.593	10.535
Gender	0.068	0.634	0.388	1.034				
Age of Jaundice	0.023	1.208	1.026	1.422				
Age of Hospitalization	0.026	1.096	1.011	1.188				
Age of Peak Total Bilirubin	0.072	1.071	0.994	1.154				
Birth Weight	0.035	0.999	0.999	1.000				
Hospitalization Weight	0.070	0.999	0.999	1.000				
HGB	0.000	1.021	1.010	1.032				
RBC	0.000	2.379	1.619	3.496				
DAT(±)	0.197[a]	0.367	0.080	1.686	0.015[a]	0.106	0.018	0.646
DAT(1+)	0.004[a]	0.356	0.175	0.722	0.004[a]	0.280	0.116	0.674
DAT(2+)	0.001[a]	0.201	0.080	0.509	0.001[a]	0.152	0.049	0.472

[a] Compared with DAT negative

Discussion & Conclusions

The great variation of the distribution of ABO and RhD blood groups has led to distinct distribution of types of HDN throughout the world. A, O and RhD + were the most frequent phenotypes in ABO and RhD

blood groups respectively in mainland China. In 2012, Guo N's study[18] enrolled 512594 donations at five blood centers in China reported that O phenotype was the most frequent (34.0%) and RhD– phenotype was 1.0%, in 2017, Liu J's research showed that blood group A (30.4%) and O (30.2%)

appeared to be the most common phenotype and RhD– phenotype was 0.95% among 3473527 Han ethnicity from China[19]. However, the proportion of RhD– phenotype has

been reported to be 14.6% in the USA[20], 17.9% in Sweden and Denmark[21], therefore, in mainland China, researches on HDN mainly focus on ABO-HDN rather than Rh-HDN compared with HDN studies on Caucasian populations. Meanwhile, East Asian race with ABO-HDN has higher risk of severe hyperbilirubinemia[22] which may lead to acute and chronic nervous system damage with no early intervention. In severe ABO-HDN cases, transfusions of blood components were required usually to reduce jaundice, prevent and treat anemia, however, blood components transfusion may lead to transfusion transmitted diseases, anaphylaxis, hypersensitivity, thrombosis, electrolyte disturbance and renal failure as well as high hospitalization cost.

The incidence of positive DAT in ABO-HDN ranges greatly due to the different DAT techniques and population examined, in this study, the incidence of positive DAT is 25.6% (69/270), previously, Valsami S et al retrospectively reviewed routinely performed DAT of all infants born between January 2011 and December 2012 in Greece showed that, among 481 ABO incompatible infants, 64 cases are confirmed as DAT positive (positive rate is 13.3%)[23] and a 2-year retrospective study with a large sample size of ABO-incompatible neonates of black ethnicity showed that 270 of 1537 cases are DAT positive with the positive rate of 17.6%[24] The incidence of positive DAT is higher than that of previous reports, in addition to race differences, that DAT was used as screening test to perform in all cases with suspected ABO-HDN instead of infants with confirmed ABO-HDN in our study, could be the main reason cause of this difference, hence, even if the positive rate of DAT has risen with the increase of the possibility of ABO-HDN in the study population, the incidence of positive DAT in ABO-HDN is still low, illustrating DAT a poor positive predictor of ABO-HDN. Moreover, we investigated the clinical value of DAT in severe hyperbilirubinemia in the present study, although ABO-HDN refers to be the major reason of positive DAT, whether DAT could be a prediction factor for severe hyperbilirubinemia is still controversial, some studies reported that the positive DAT has only a poor predictive value for severe hyperbilirubinemia because of only approximate 23% cases of ABO-HDN with positive DAT will continue to have significant hyperbilirubinemia[25,26], however, this study shows that DAT has a predictive value for the severe ABO-HDN hyperbilirubinemia with the DAT level cutoff value of ± has the highest sensitivity and specificity in terms of accurately predicting severe ABO-HDN hyperbilirubinemia, Mehta R's study[14] enrolled 901neonates with gestational age > 34 weeks and birth weight > 2000 g showed that the risk for hyperbilirubinemia requiring phototherapy in the DAT positive infants is significantly higher than that in the neonates with negative DAT (OR 6.78, 95% CI 2.38–19.33) and previous studies demonstrated that ABO-HDN with a positive DAT is considered a major risk factor for the development of severe hyperbilirubinemia and neurotoxicity[27,28] as well.

The combination detection[29] of DAT and detection factors including the blood cell indices, pre-discharge total bilirubin level, cord serum albumin and cord bilirubin/albumin ratio[30] would be more beneficial than that of DAT alone for predicting the severity of ABO-HDN. Among them, cord serum albumin and cord bilirubin/albumin ratio have been well researched as the result of the mechanism of bilirubin metabolism in infants, in plasma, bilirubin binds to albumin to form the bilirubin albumin complex in order to transport to the liver for further metabolism, on one hand, bilirubin binding to albumin increases the water solubility of bilirubin and improves the plasma capacity of transporting bilirubin, on the other hand, it limits the free permeability of bilirubin to various cell membranes and avoids toxic effect of bilirubin on tissues and cells[31,32], therefore, low serum albumin level decreases bilirubin clearance and thus increases significant hyperbilirubinemia, previous studies[33,34] found that term infants cases with low cord albumin < 2.8 g/dl developed more significant hyperbilirubinemia requiring phototherapy and exchange transfusion and Khairy MA and colleagues[33] showed that neonates with cord bilirubin/albumin ratio < 0.61 were at risk of developing significant hyperbilirubinemia needing interventions at the same time. However, both cord serum albumin level and cord bilirubin/albumin ratio are considered to reflect ability of albumin to bind bilirubin, AGR we assessed in the current study reflect the infant level of albumin remaining after binding bilirubin resulting in that high level of bilirubin may lead to decline of albumin level. Moreover, since this is the first analysis assessing the clinical value of AGR in severe ABO-HDN hyperbilirubinemia to the best of our knowledge, we plotted an ROC curve analysis and showed that AGR cutoff value < 2.05 had a good predictive value with a sensitivity of 54.1% and a specificity of 85.7%, meanwhile, we also demonstrated that combination detection of DAT and ARG had a better predictive value than that of respective detection of DAT and ARG in prediction of severe ABO-HDN hyperbilirubinemia (AUC for combination detection 0.750 VS AUC for DAT 0.621 and AUC for AGR 0.740). Finally, after dividing the infants into three groups according to the results of DAT and ARG and comparing phototherapy day and hospitalization day of the three groups, we found that group of AGR < 2.05 and DAT > ± had significant longer phototherapy day and hospitalization day than that of group of AGR < 2.05, group of DAT > ± respectively, prompting that the condition of infants with AGR < 2.05 and DAT > ± is more severe and meaning more hospital costs. With lack of studies done on ARG as a prediction factor of severe ABO-HDN hyperbilirubinemia, this work opens the window for further studies to be performed in this field and we are aware that larger scale trials including Multi ethnic researches and preterm neonates are needed.

To summarize, in this study DAT and AGR proved to predict the severity of ABO-HDN hyperbilirubinemia in term neonates. Infants with either DAT > ± or AGR < 2.05 were at risk of developing significant ABO-HDN hyperbilirubinemia. These can be considered possible predictors for severe ABO-HDN hyperbilirubinemia and combination detection of DAT and ARG can improve the predictive value. We recommend that pediatricians pay close attention to the results of DAT and AGR when judging the severity of ABO-HDN hyperbilirubinemia, particularly the term infants with DAT > ± and AGR < 2.05.

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Abbreviations

1. DAT
2. direct antiglobulin test
3. AGR
4. albumin globulin ratio
5. HDN
6. hemolytic disease of the newborn
7. IVIG
8. intravenous immunoglobulin
9. RBC
10. red blood cell
11. AUC
12. area under the curve
13. ROC
14. receiver operating characteristic

Declarations

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Competing interests statement:

The authors have no relevant financial or non-financial interests to disclose.

Author Contribution:

Duan Ling, Chen Ping, Tu Na and Hu hongbing contributed to the study conception and design, Duan Ling, Chen Ping and Tu Na performed material preparation, data collection and analysis, Duan Ling wrote

the first draft of the manuscript text, Duan Ling and Chen Ping prepared table1-2 and figures1-3. Hu Hongbing guided the process of the whole research, including revised the manuscript text and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology and because this is an observational study using the research of medical records obtained in previous clinical diagnosis and treatment, the Ethics Committee of Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology has confirmed no consent to participate statement is required.

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Figures

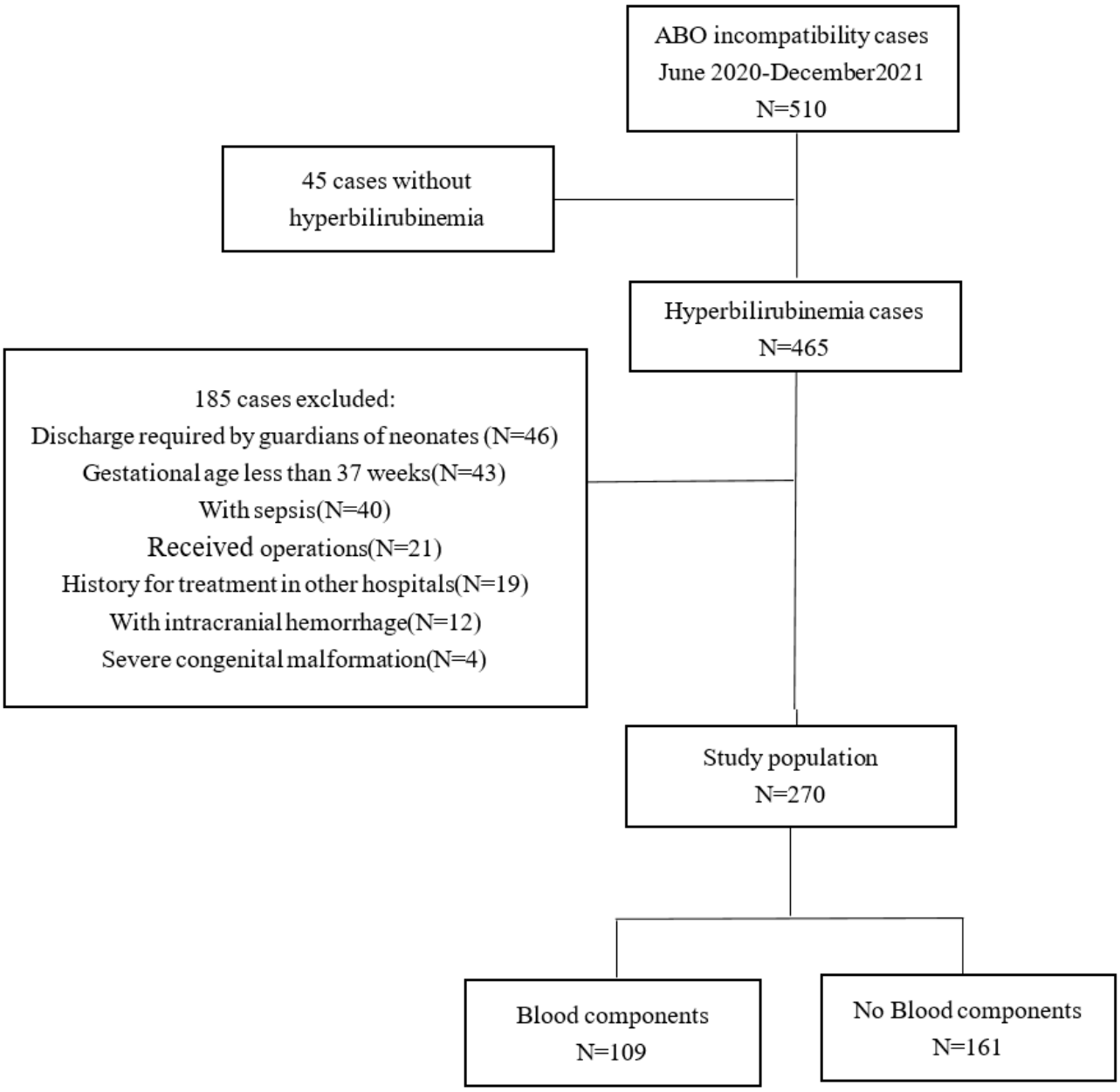

Figure 1

Flowchart of the study population

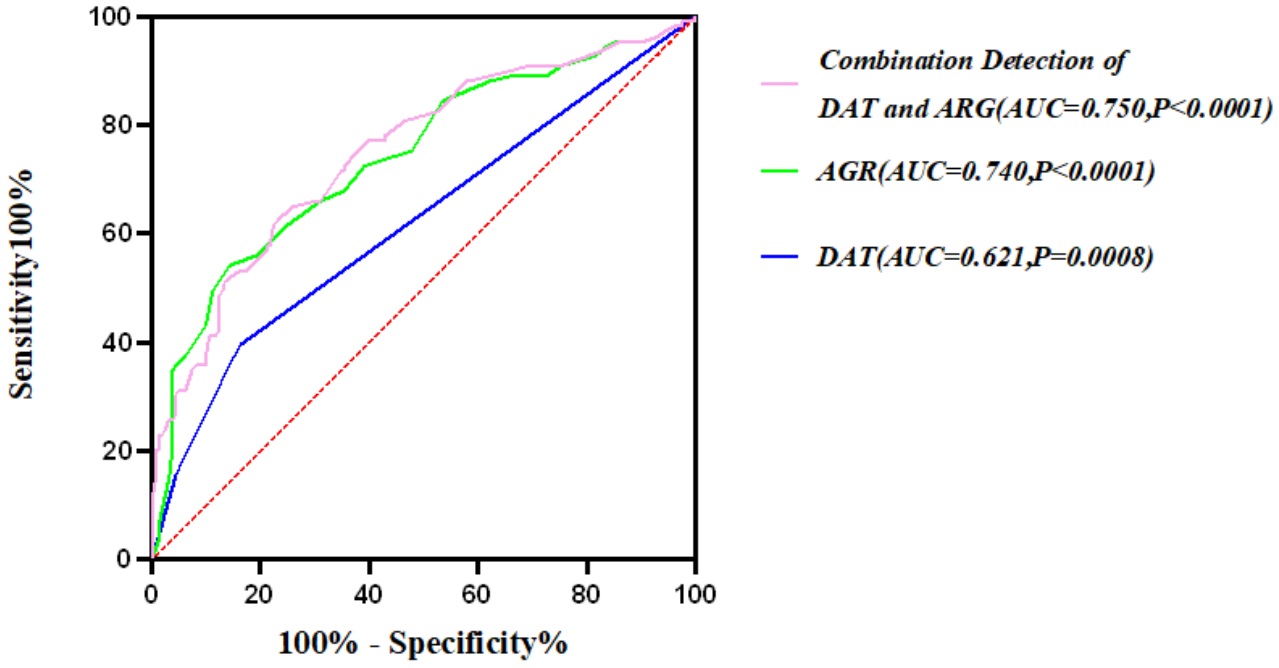

Figure 2

ROC curves of DAT, AGR and combination detection of DAT and ARG

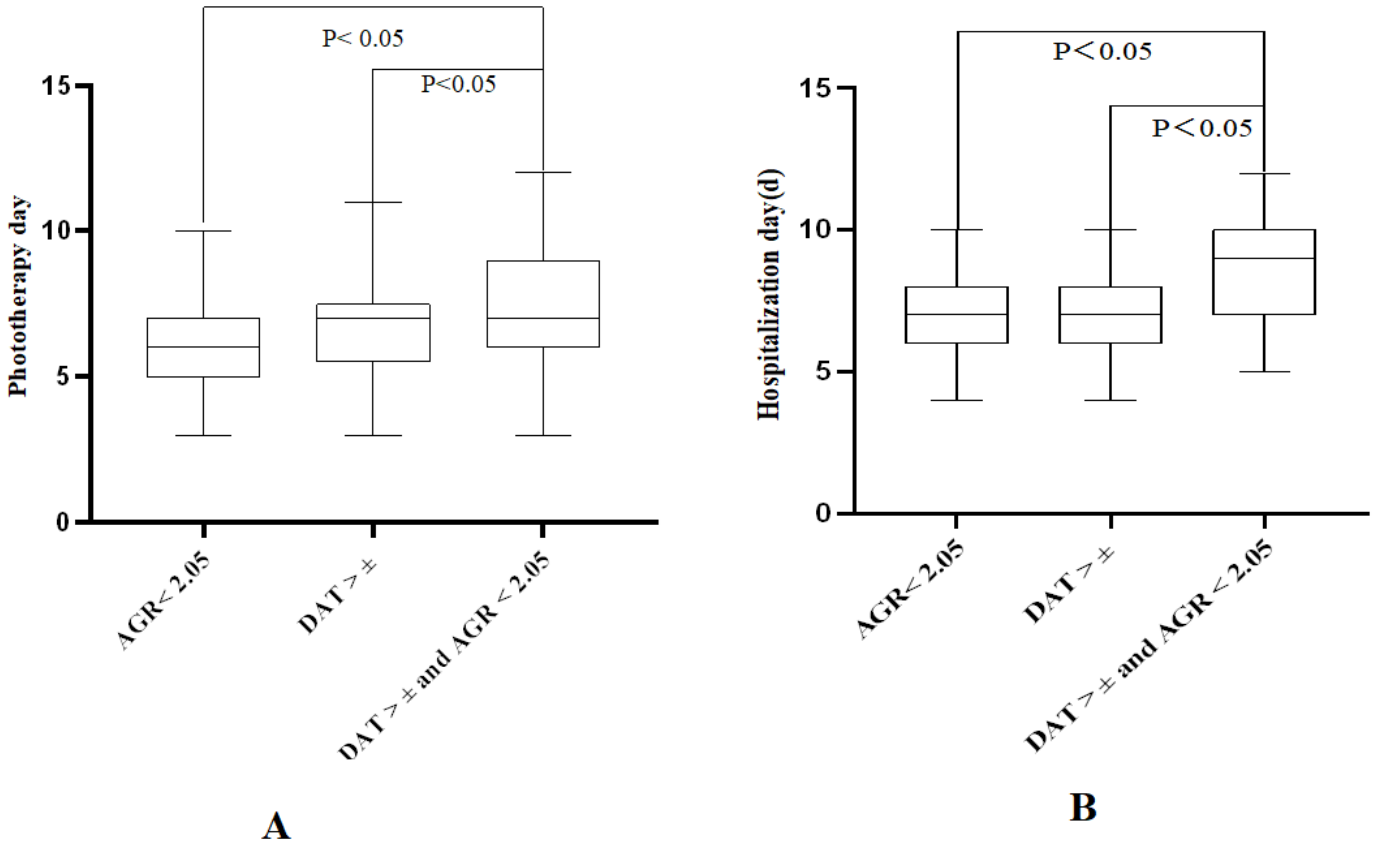

Figure 3

(A). The difference of phototherapy day of three groups

(B). The difference of hospitalization day of three groups