Different distribution of maternal red cell antibodies and risk on the severe alloimmune haemolytic disease of the foetus in a Chinese Population: a retrospective cohort study on prenatal management

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Abstract

BACKGROUND: The distribution of antibodies that cause haemolytic disease of the foetus (HDF) in China and the effects of multiple antibodies on the severity of HDF are needed to further evaluation.

METHODS: A retrospective cohort study was conducted in two affiliated Hospitals of Sun Yat-sen University. Total 268 pregnant women and foetuses pairs were divided into four groups: anti-D, anti-D combined others, other single-antibody and other multiple-antibody. The obstetrics history, the incidence of severe HDF and foetal outcomes were collected and compared in each group. The logistics regression analysis and the survival analysis were conducted for analysis.

RESULTS: Anti-D was the most common cause of HDF, followed by the anti-M of MNS system. No anti-K or isolate anti-c associated HDF was found. Upon the mothers with anti-D combined other antibodies, the incidence of severe HDF was increased than those with anti-D alone(P=0.046). Foetuses in the other single-antibody group had lower reticulocytes (P=0.007), more IUT times (P =0.007) and an earlier onset of severe HDF (P=0.003). High maternal antibody titer (P□0.001), multiple affected pregnancies (P□0.001) and other single-antibody (P=0.030) were the independent risk factor for HDF developing. And the lower foetal haematocrit (P =0.012) and higher reticulocyte counts (P =0.035) were the independent risk factors of severe HDF in the anaemia foetuses affected by RhD alloimmunization.

CONCLUSION: The distribution of HDF-associated antibodies in China is different from that in western countries. Other single non-Rh(D) and multiple antibodies could both increase the risk of HDF but anti-D combined with other antibody would not influence the severity of the disease than anti-D alone.

Background

Haemolytic disease of the foetus and new-born (HDFN) is the most common aetiology of haemolytic anaemia in foetuses and hyperbilirubinaemia in neonates[1]. As the wide prophylactic use of anti-D immunoglobulin has greatly decreased the incidence of Rh(D) alloimmunization in western countries[2], the incidence of HDFN caused by non-Rh(D) antibodies has increased [3, 4]. However, the incidence of anti-D-related HDFN still contributes to the morbidity and mortality of foetuses and new-borns in China[5]. A total of 60.8% of haemolytic disease is caused by anti-D, followed by anti-E, anti-c and the antibodies in the MNS system, during the neonatal period[6, 7]. Thus far, the distribution of non-ABO antibodies that cause haemolytic disease of the foetus (HDF) in China is not well known. In addition, some women have multiple red cell antibodies, which might lead to more complicated situation than a single red cell antibody during pregnant management. Some studies have found that foetuses with multiple antibodies need a more interventions than those affected by only anti-D[8]. Thus, the objective of our study was to characterize the distribution of antibodies that cause HDF and to evaluate the effect of different antibodies on the severity of HDF in a Chinese population.

Methods

Study population

This was a retrospective two-centre cohort study including pregnant women with non-ABO red cell alloimmunization and their foetuses in the First Affiliated Hospital and the Sixth Affiliated Hospital of Sun Yat-sen University from January 2005 to May 2019. All patients provided written informed consent for each medical intervention. The Guangzhou Blood Centre identified the antibodies responsible for red cell alloimmunization. We included only women with immunoglobin G (IgG) red cell antibody in the study. The antenatal diagnostic criteria for HDF were as follows: 1) the detection of non-ABO IgG antibodies in maternal serum and corresponding to maternal-foetal blood group incompatibility; 2) foetal anaemia confirmed by cordocentesis or a relative adverse pregnancy outcome; and 3) a positive antibody elution test from foetal red blood cells (RBCs), which was the direct evidence of causing haemolysis. In cases of missing values, treatment with intrauterine transfusion (IUT), hydrops foetalis and foetal demise were also considered as valid confirmation of clinically relevant HDF. We excluded women and their foetuses based on the following criteria: 1) the woman underwent termination of pregnancy due to foetal structure or chromosome abnormalities; 2) the woman with alloimmunization had the same blood group phenotype as that of her foetus; 3) the woman had unknown antibodies; or 4) the woman was loss to follow-up. Stillbirth cases in this study did not include foetal death due to the discontinuation of treatment and induction of labour. Not all the data were available for each case.

Data collection

Data on the maternal obstetric history, transfusion history, type of alloimmunization, presence or absence of hydrops, and foetal sex were collected. For the anaemia foetuses, we further collected data on the gestational age at diagnosis, foetal haemoglobin, haematocrit, reticulocyte count, reticulocyte percent before IUT, number of IUTs and foetal outcomes. Women who underwent multiple times of antibody identification during the same pregnancy were included as a single entry. Regarding the women who were pregnant more than once during the study period, each pregnancy was included in our report. To determine the effects of different alloimmunizations, the patients were classified into four groups according to their antibodies, namely, only anti-D (anti-D group), anti-D combined with other antibodies (anti-D combined others group), other single antibody (other single-antibody group) and other multiple antibodies combinations (other multiple-antibody group).

Definition and treatment policy

Foetal anaemia was confirmed by cordocentesis. Severe foetal anaemia and the indication for IUT were as previously described[9, 10].

Primary and secondary outcome

The primary outcome was the occurrence of severe HDF, which was defined as severe foetal anaemia, hydrops fetalis, stillbirth, or the need for IUT due to maternal alloimmunization[1]. The secondary outcomes were the number of IUTs, the severity of the foetal anaemia and the outcomes of the foetuses.

Statistics analysis

Statistical analysis was performed using SPSS 22.0 statistical software. Quantitative variables were expressed as medians with 25th and 75th quartiles or means with their standard deviations. Chi-square tests or Fisher's exact tests were used for distribution-based comparisons between groups. The t-test was used when the values were normally distributed, while the Mann-Whitney U test was applied when the values were abnormally distributed. The risk factor of severe HDF in foetuses with maternal alloimmunization were analysed by the logistics regression analysis. The results are presented as p values and a two-sided p value < 0.05 was regarded as statistically significant. The odds ratios (OR) with 95% confidence intervals (95% CIs) were also presented. A Kaplan-Meier curve of survival analysis depicting the severe HDF-free interval is presented. The Log Rank method was used for the survival interval comparison between groups. The survival curve was calculated by GraphPad Prism 5.0.

Results

From January 2005 to December 2019, a total of 390 pregnant women with positive alloantibodies. Approximately 122 cases were excluded from our study based on the criteria as follows: loss to follow-up (n = 110), termination of pregnancy due to foetal structure abnormalities (n = 4), specific maternal-foetal blood phenotype compatibility (n = 6) or unknown antibodies (n = 2) (Fig. 1). A total of 268 pregnant women with non-ABO alloantibodies and their 268 foetuses were finally included in our study.

Basic characteristics

The average age of the included pregnant women was 31.1 ± 4.3 years old (the ages of 10 cases were unknown). Approximately 15 women had more than one pregnancy during the study period and thus were represented more than once in our dataset. The patients in this cohort were divided as follows: anti-D group (n = 203), anti-D combined others group (n = 38), other single-antibody group (n = 24) and other multiple-antibody group (n = 3). The anti-D group had the most women with a history of blood transfusion. The basic information of the four groups is shown in Table 1. The women in anti-D combined others group had significantly more gravidities (P = 0.002) and higher rate of previous affected history of HDFN (P = 0.011) than the anti-D group (Table 1).

Table 1
Baseline data between Groups

	Anti-D n = 203	Anti-D combined others n = 38	Other single-antibody n = 24	Other multiple-antibody n = 3
Maternal age (years)	31.5 ± 4.3 (Unknown = 11)	30.5 ± 4.5	29.3 ± 4.2	29.0 ± 3.6
P		0.368	0.125	0.434
Gravidity (times)	3 (2–4) (Unknown = 9)	4 (3–5)	3 (2-3.8)	5 (2.1-5)
P		0.002	0.262	0.373
1	8	1	3	0
2	53	4	6	1
≥2	133	33	15	2
Maternal transfusion history n(%)	30 (14.8)	7 (18.4)	2 (8.3)	1 (-)
P		0.568	0.543	0.389
Women with previously affected history n(%)	80(39.4) (Unknown = 7)	24 (63.2)	14 (58.3)	2 (-)
P		0.011	0.075	0.570
Foetal sex				
Female n	89	18	6	1
Male n	100	20	15	1
Unknown n	14	0	3	1

The incidence of HDF among cases with different types of antibody

Approximately 45.5% (122/268) of foetuses suffered from HDF, and 34.7% (93/268) had IUT before delivery. Anti-D was the most common causal antibody (82/122, 67.2%) of HDF, and 78.0% (64/82) of patients underwent IUT (Tables 2). The cases of single antibody included anti-M (n = 18), anti-E (n = 3) and anti-Mur (n = 3). And the cases of multiple antibodies included anti-Ec (n = 2) and anti-Ce (n = 1). The anti-M of the MNS system was the second common antibody other than the Rh system.

Table 2

The incidence of severe HDF between different types of antibody

	Anti-D n = 203	Anti-D combined others n = 38	Other single-antibody n = 24	Other multiple-antibody n = 3
HDF n(%)	82 (40.4)	23 (60.5)†	15 (62.5) †	2 (-)‡
P		0.022	0.038	0.568
Severe HDF* n(%)	72 (35.5)	20 (52.6) †	15 (62.5) †	2 (-)‡
P		0.046	0.010	0.293
Severe anaemia n(%)	38 (18.7)	9 (23.7)	8 (33.3)	0
P		0.478	0.307	> 0.999
Hydrops foetalis n(%)	18 (8.9)	3 (7.9)	8 (33.3) †	0
P		> 0.999	0.002	> 0.999
Intrauterine demise n(%)	11 (5.4)	2 (5.3)	3 (12.5)	1 (-)‡
P		> 0.999	0.173	0.166
IUT n(%)	64(31.5)	18 (47.4)	10 (41.6)	1 (-)‡
P		0.059	0.316	> 0.999
The survival rate of HDF				
Foetuses	55(67.1)	18 (78.3)	12(80.0) †	1 (-)‡
P		0.303	0.380	> 0.999
Neonates	55(67.1)	18 (78.3)	9 (60.0)	1 (-)‡
P		0.303	0.595	> 0.999

HDF: the haemolytic disease of the foetus;IUT□intrauterine transfusion□

* Foetuses may fall into more than 1 severe HDF defining categories;

† Significant difference compared to the anti-D group;

‡ Can't calculate the percentage due to denominator less than 20.

Table 3
The risk factors of HDF in foetuses with maternal alloimmunization

	HDF (n = 122)	No HDF (n = 146)	P	Univariable OR (95% CI)	P	Multivariable OR (95% CI)
Previous affected pregnancies per women *	1(0–2)	0 (0–0)	0.001	5.718 (3.599–9.083) †	0.001	5.392 (3.217–9.039) †
Maternal transfusion history, n (%)	19(15.6)	21(14.4)	0.785	0.911 (0.465–1.785)	0.013	4.255(1.362–13.299) †
Maternal antibody titre*	1:512(1:256-1:1280)	1:64(1:8 – 1:256)	0.001	1.002(1.001–1.002) †	0.001	1.002(1.001–1.003) †
Types of antibody						
Anti-D, n (%)	82(67.2)	121(82.9)	-	-	-	-
Anti-D combined others, n (%)	23(18.9)	15(10.3)	0.024	2.263(1.114–4.594) †	0.806	0.874(0.297–2.573)
Other single-antibody, n (%)	15(12.3)	7(4.8)	0.043	2.459(1.028–5.886) †	0.030	3.559(1.131–11.199) †
Other multiple-antibody, n (%)	2(1.6)	1(0.7)	0.568	2.951(0.263–33.082)	0.763	0.387(0.001-184.979)
HDF: haemolytic disease of the foetus						
* Data present as Medium (25th – 75th quartiles)						
† Significant difference						

Furthermore, different types of antibodies showed the various risk of HDF. The incidence of severe HDF of the anti-D combined others group was significantly higher than that of the anti-D group (52.6% vs. 35.5%, P = 0.046). The need for IUT in the anti-D combined others group tended to be higher than that in the anti-D group (47.4% vs. 31.5%, P = 0.059). The other single-antibody group, had a significantly higher incidence of HDF (62.5% vs. 40.4%, P = 0.038), hydrops fetalis (33.3% vs. 8.90%, P = 0.002) and severe HDF (62.5% vs. 35.5%, P = 0.010) than the anti-D group (Table 2). The anti-M associated risk for severe HDF (10/18, 55.6%) was similar to that of anti-D(72/203, 35.5%, P = 0.091).

There were 17 cases of intrauterine demise, including 11 cases due to the anti-D, 2 cases due to the anti-D with anti-C, 2 cases due to the anti-M, 1 case due to anti-Mur and 1 case due to the anti-Ec. Nine of these 17 cases did not survive after IUT. The other 8 cases discontinued treatment and terminated pregnancies because of severe foetal hydrops or severe foetal anaemia. Three cases were delivered by emergency

caesarean but finally died in the neonatal period. The survival rate of HDF was no difference between the 4 groups.

The risk factors of HDF in the foetuses with maternal alloimmunization

In the univariable analysis among the foetuses with maternal alloimmunization, four variables were associated with the occurrence of HDF: more previous affected pregnancies per women(OR 5.718, CI 3.599–9.083), higher maternal antibody titre (OR1.002, CI 1.001–1.002), anti-D combined others compared with anti-D(OR 2.263, CI 1.114–4.594); Other single-antibody compared with anti-D(OR 2.459, CI 1.028–5.886); There were no statistical differences between HDF and non-HDF foetuses for maternal blood transfusion history or other multiple-antibody. In multivariable analysis, there were four variables associated with the occurrence of HDF: more previous affected pregnancies (OR 5.392, CI 3.217–9.039), maternal transfusion history (OR 4.255 CI 1.362–13.299), higher maternal antibody titre (OR 1.002, CI 1.001–1.003), other single-antibody compared with anti-D (OR3.559, CI 1.131–11.199).

Foetal anaemia among different types of antibody

Among the foetuses suffering from HDF, there were six cases of foetal demise before cordocentesis (3 cases with anti-D, 2 cases with anti-M and 1 case with anti-Ec). The pregnancies were terminated in two cases (1 case with anti-Mur and 1 case with anti-M) because of severe foetal hydrops without cordocentesis. One case (anti-DC-related HDF) had an emergency caesarean at 34^{+5} weeks of gestation because of foetal distress and a rapid increase in antibody titre. Therefore, a total of 113 foetuses with haemolytic anaemia confirmed by cordocentesis were included in the analysis. The distribution of antibody that resulted in foetal haemolytic anaemia is shown in Table 4. There was no significant difference in foetal anaemia degree distribution among the four groups(P = 0.466). The antibodies of the Rh blood group system were the most common antibodies leading to foetal anaemia (92.0%,104/113), followed by the antibodies in the MNS blood group system, including anti-M (n = 7) and anti-Mur(n = 2). Anti-D was the most common antibody to cause foetal anaemia. It was effective alone (79/113,69.9%) or in combination with anti-C (15 cases) or anti-E (7 cases) (22/113, 19.5%) in causing foetal haemolytic anaemia. Anti-E caused one case of severe anaemia, one case of mild anaemia and one case of moderate anaemia with anti-c, respectively.

Table 4
The different degrees of foetal haemolytic anaemia according to the types of antibody

	Anti-D	Anti-D combined others	Other single-antibody	Other multiple-antibody	Total
Mild	31	6	2	0	39
Moderate	10	6	1	1	18
Severe	38	10	8	0	56
Total	79	22	11	1	113

Regarding the characteristic of foetuses with anaemia and their mothers, those who had anti-D combined with other antibodies had more pregnancies affected by HDFN than those who had anti-D alone [1 (0–1) vs 2 (1–2), P = 0.005]. However, there was no significant difference in the foetal haemoglobin concentration, haematocrit, reticulocyte count and percentage, or gestational age at birth between these two groups (Table 5). The maximal antibody titre was significantly lower in the other single-antibody group [1:32 (1:8 − 1:256)] than in the anti-D group [1:512 (1:512-1:2048)] (P = 0.001). Besides, the foetuses in the other single-antibody group had a significantly lower reticulocyte count (142.1 ± 122.6 × 10^9/L vs 303.0 ± 97.3 × 10^9/L, P = 0.007) and reticulocyte percent [8.5 (4.2–17.1)% vs. 15.7 (10.1–22.8)%, P = 0.015] but a higher number of IUTs [5 (3–5) vs 2 (1–4), P = 0.007] than the anti-D group (Table 5).

Table 5

Characteristics of anaemia foetuses between three groups

	Anti-D n = 79	Anti-D combined others n = 22	P *	Other single-antibody n = 11	P *
Previous affected pregnancies per women	1(0–1)	2(1–2)	0.005	1(0–1)	0.990
Antibody titre	1:512 (1:512-1:2048)	1:512 (1:256-1:2048)	0.464	1:32 (1:8 – 1:256)	0.001
Haemoglobin(g/L) †	68.4 ± 26.6	67.7 ± 2.6	0.908	50.2 ± 23.1	0.033
Haematocrit (%)†	20.9 ± 7.7	20.5 ± 6.3	0.831	15.2 ± 6.6	0.023
Reticulocyte count(× 10^9/L) †	303.0 ± 97.3§	331.3 ± 104.9	0.245	142.1 ± 122.6‖	0.007
Reticulocyte percentage (%)‡	15.7 (10.1–22.8) §	9.9(13.6–31.3)	0.168	8.5 (4.2–17.1) ‖	0.015
Hydrops, n (%)	15(19.0)	3 (13.6)	0.756	3 (33.3)	0.235
Number of IUTs§	2(1–4)	3.5 (1–5)	0.174	5 (3–5)	0.007
Gestational age at birth c	35.0 (33.9–36.6)	36.0 (33.4–36.9)	0.586	35(33.2–36.0)	0.331

IUT: intrauterine transfusion;

* Significant difference compared to the anti-D group

† Data presented as Mean ± SD

‡ Data present as Medium (25th – 75th quartiles)

§ Assessed in 72/79(91.1%)foetuses,missing value for 7 cases;

‖Assessed in 6/9(66.7%)foetuses,missing value for 3 cases;

Table 6

The predictors of severe HDF in foetuses with anti-D and/or combined other antibodies

	Severe HDF (n = 90)	No severe HDF (n = 11)	P	Univariable OR (95% CI)	P	Multivariable OR (95% CI)
Female, n (%)	42 (46.7)	7(63.6)	0.446	0.596(0.157–2.257)	0.522	0.398(0.024–6.698)
Maternal antibody titre *	1:512(1:512-1:2048)	1:768 (1:256-1:2560)	0.067	1.001 (1.000-1.003)	0.999	0.999 (0.998–1.001)
Diagnostic gestation age, weeks *	27.9(24.0-32.7)	32.4 (32.7–34.0)	0.015	0.724 (0.558–0.940) ‡	0.462	1.150 (0.793–1.667)
Haematocrit†, %	19.7 ± 7.0	30.2 ± 2.1	0.002	0.000 (0.000–0.000) ‡	0.012	0.000(0.000–0.000) ‡
Reticulocyte count †(10^9/L)	318.4 ± 99.8	226.7 ± 59.4	0.012	1.013 (1.003–1.022) ‡	0.035	1.030(1.002–1.059) ‡
Anti-D combined others, n(%)	21 (23.3)	1 (9.1)	0.359	2.700 (0.323–22.556)	0.387	5.365(0.119-241.983)
HDF: haemolytic disease of the foetus; OR: odds ratio;						
* Data present as Medium (25th − 75th quartiles)						
† Data presented as Mean ± SD						
‡ Significant difference						

The risk factors of severe HDF in foetuses with Rh(D) alloimmunization

Among foetuses who affected by anti-D, including those combined with other antibodies,three variables were associated with the occurrence of severe HDF: the diagnostic gestation age (OR 0.724; CI 0.558–0.940), foetal haematocrit (OR 0.000 CI 0.000–0.000) and reticulocyte count (OR 1.013 CI (1.003–1.022). In multivariable analysis, two variables were associated with the occurrence of severe HDF: foetal haematocrit (OR 0.000 CI 0.000–0.000) and reticulocyte count (OR 1.030 CI 1.002–1.059).

Severe HDF-free survival

Approximately 45.5% (122/268) of the foetuses in our study were affected by maternal alloimmunization and developed HDF. We performed a survival analysis by Kaplan-Meier curve to determine the gestational

week intervals of non-severe HDF (Fig. 2). As the only 2 cases in other multiple-antibody group were from the same pregnant women, we didn't include these 2 cases into the analysis. The median survival time of non-severe HDF between these three groups was a significant difference (P = 0.023). In the anti-D group, 12% of foetuses did not develop severe HDF (Table 2). The median survival time of non-severe HDF was 29.6 weeks, and the longest gestational weeks of non-severe HDF was 34.7 weeks in this group. When compared to the anti-D combined others group, the median survival time was no significant difference (29.6 weeks vs. 26.7 weeks, P = 0.637). But the median survival time of non-severe HDF in the other single-antibody group was significantly shorter than that in the anti-D group (26.1 weeks vs. 29.6 weeks, P = 0.003), suggesting that the foetuses in the other single-antibody group might be affected by maternal alloimmunization earlier.

Discussion

The distribution of maternal alloimmunization and HDFN varies from different countries and population[11, 12]. Thus far, the antibody distribution of HDF antibodies in China has not been reported. In our study, we analyzed 268 pregnant women with red cell alloimmunization and their foetuses, including 122 foetuses with haemolytic disease, to characterize the antibody distribution of HDF in a Chinese population. Anti-D was still the most common cause of HDF (67.2%). However, in our study no case of HDF due to isolated anti-c was found, which was the secondary cause of severe HDF after anti-D antibody in the Caucasian population[4]. The difference might be due to the significantly lower frequency of c antigen in the Chinese population than in the Caucasian population[11]. Similarly, there was no case of anti-K HDF or woman who was positive for the anti-K antibody. As the extremely low frequency of K antigen of the Kell blood group system in the Chinese population, anti-K antibody positivity is extremely rare[11], and anti-K-related HDN has scarcely been reported[13]. Therefore, the distribution of HDF-associated antibodies in the Chinese population is different from that in the Caucasian population.

Moreover, we found that anti-M was the most common non-Rh(D) antibody, with a similar incidence of HDF (10/18, 55.5%). There were very few reports that causally link anti-M antibody positivity with HDFN in a Caucasian population[14]. Over the past two decades, anti-M-related HDFN has mostly been reported in the Asian ethnic group, especially in Japanese[15] and Chinese populations[16, 17], resulting in severe foetal anaemia, hydrops fetalis, stillbirth and neonatal death. The previous study found that approximately 88.6% of anti-M-related HDFN cases occurred in the Asian population[17], indicating the high pathogenicity of IgG anti-M in the Chinese people. Even though our tertiary prenatal centre provided a relatively higher incidence of HDF due to anti-M, our results suggested that anti-M might have a significantly high risk for severe HDFN in the Chinese population, with 60.0% (6/10) receiving IUT and 2 cases of intrauterine demise due to lack of timely treatment. The lower reticulocyte count indicated incompatible erythropoiesis with severe anaemia,[17] which was also found in neonatal cases[15]. These might be explained by the suppression of red cell development from the erythroid precursor cell[18, 19]. As a consequence, the anti-M related HDFN can have a negative result of direct anti-human globulin test (direct Coombs test) [15], making the diagnosis of anti-M-related HDFN to be difficult and frequently missed.

Multiple antibodies seemed to increase the risk of HDF-associated morbidity without influencing the severity of foetal anaemia. The incidence of severe HDF and IUT became significantly higher when the pregnant women had anti-D combined with other antibodies, which was consistent with the previous studies [20–22]. However, after the logistics regression analysis, anti-D combined with other antibody was not an independent risk factor for HDF. What's more, among the anaemia foetuses, no significant difference was found in the foetal haemoglobin concentration or gestational age at diagnosis between the anti-D group and the anti-D combined others group. The similar level of the reticulocyte count and percentage between these two groups indicated similar haematopoiesis conditions. Some studies reported that neither the foetal anaemia severity nor gestational age at first IUT was influenced by anti-D with addition antibodies[8, 23]. In the logistics regression analysis of anaemia foetuses affected by Rh(D) alloimmunization, anti-D combined with other antibody was not a risk factor for severe HDF neither in univariant analysis nor multivariant analysis. These results indicated the high incidence of HDF in anti-D combined with others group was mixed with other confounding factors. Furthermore, we also found that women with multiple antibodies had a higher rate of previous HDFN affected history and more affected times than those with only anti-D. These results might suggest that the more often an Rh(D)-negative woman expose to an Rh(D) incompatible foetus, the higher chances of generating additional antibodies, leading to a more aggressive immune response and cumulative effect towards increasing the risk of haemolysis and developing severe HDF. As anti-D still played a dominant role in haemolysis, the severity of HDF was not significantly different regardless of whether anti-D was present alone or in combination with other antibodies due to the same alloimmunization. Therefore, when a pregnant woman has multiple antibodies, her foetus might have a higher risk of developing severe HDF and should be closely monitored during the antenatal period.

In the regression analysis, we found that high maternal antibody titre, more previous affected pregnancies, and other single-antibody were the independent risk factors for the occurrence of HDF. Even though in some alloimmunization, low antibody titre can cause severe HDFN[24], but high titre suggested a more active immune response, increasing the risk of HDF. Moreover, the more affected history might recurrently stimulate a larger amount and long duration antibody[25], which can cross the placenta to cause the disease in the foetal period. In the Rh(D) alloimmunization, once the foetal anaemia occurred, the antibody titre can't predict the risk of severe HDF. The lower foetal haematocrit and higher reticulocyte count were independent risk factors of severe HDF. The severer of anaemia and more active erythropoiesis foetuses present, the severer of the disease, indicating the need of intrauterine intervention.

The Kaplan-Meier analysis to determine the severe HDF-free interval was conducted to perform a time data comparison among different antibodies, and the results provided a clinical reference to estimate the gestational age at onset of severe HDF and the applicable time for clinical intervention. The significant difference in median survival time among groups indicated the onset time of severe HDF was various from types of antibody. The other single-antibody group had significantly shorter survival interval, providing direct evidence that foetuses affected by other single-antibody group, which consists mainly of anti-M, might develop severe HDF earlier than those affected by anti-D alone. This can be explained by

the earlier development of antigens in the MNS system than in the Rh system during foetal development and erythropoiesis[26].

There were still some limitations to our study. Our centre is a tertiary prenatal care centre, and the patients therein might have a higher risk for HDFN, more severe HDFN disease, and its associated complications. Therefore, the morbidity and mortality rates might be higher among patients in this study than among individuals throughout the country. Because of the insufficient sample size of specific non-anti-D antibodies, we cannot compare the differences between specific antibodies, especially in terms of other multiple antibodies.

Conclusion

The antibody distribution for HDF in China is different from that in western countries. Multiple antibodies might increase the risk and incidence of severe HDF but might not influence the severity of foetal anaemia compared to isolate anti-D. High maternal antibody titre and multiple affected pregnancies are the independent risk factor of HDF developing.

Abbreviations

HDFN
Haemolytic disease of the foetus and new-born
IgG
Immunoglobin G
HDF
Haemolytic disease of the foetus
IUT
Intrauterine transfusion
OR
Odds ratio
CI
Confidence interval

Declarations

Ethics approval and consent to participate

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the ethics committee of the Sixth Affiliated Hospital of Sun Yat-sen University, in Guangzhou, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Authors' contributions

Si Li designed and performed the research, analysed the data and wrote the paper; Zhingming He & Yanmin Luo performed the research; Yanli Ji & Guangping Luo made contributions to alloantibody identification and analysis. Yu Gao and Qun Fang designed the research study and performed the major revisions to the paper.

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Figures

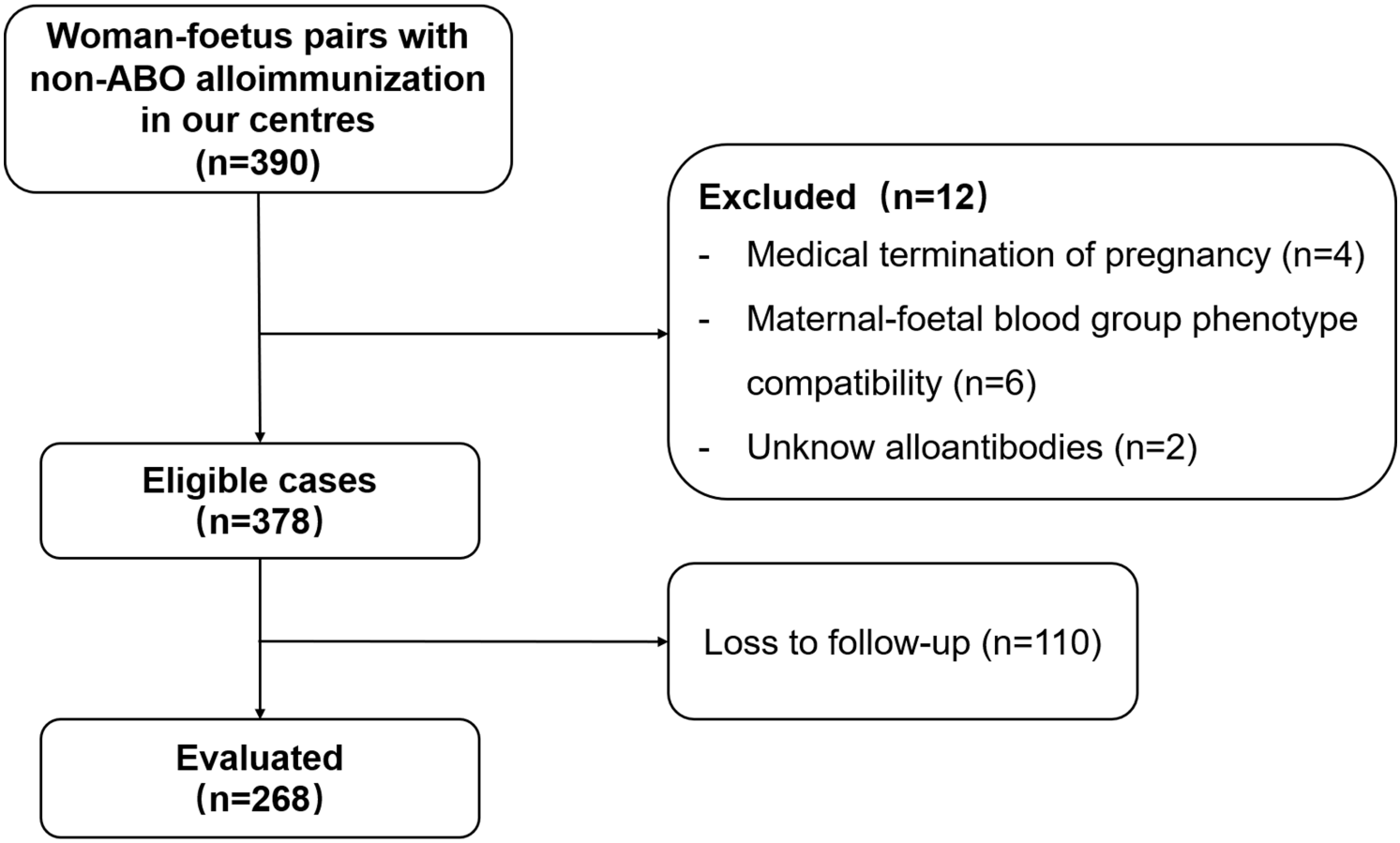

Figure 1

Flow-chart of the study participants;

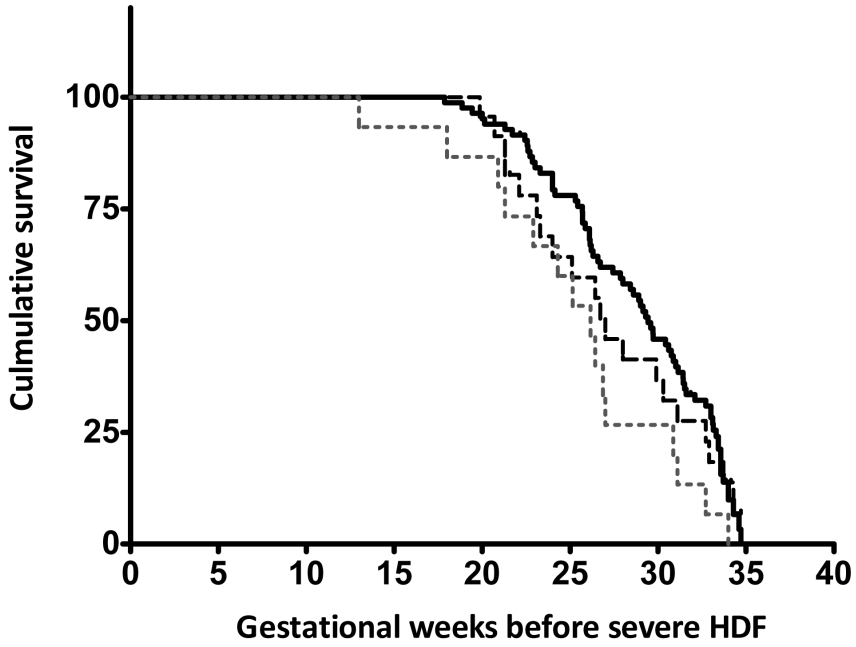

Figure 2

Kaplan-Meier curve, non-severe HDF intervals between groups; HDF: haemolytic disease of the foetus —— anti-D — anti-D combined others …… Other single-antibody