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Perinatal risk factors associated with severity of haemolytic disease of the foetus and newborn due to Rhc maternal-foetal incompatibility: A retrospective cohort study

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Abstract

Background and Objectives: Anti-c is the third red blood cell antibody responsible for haemolytic disease of the foetus and newborn (HDFN) requiring intrauterine transfusion. We aimed to identify risk factors associated with HDFN and severe HDFN due to Rhc maternal-foetal incompatibility.

Materials and Methods: A retrospective cohort study was conducted in Paris and the surrounding area (France), between 2013 and 2015. We included mothers and their children managed by the National Reference Centre in Perinatal Hemobiology for alloimmunization and maternal-foetal incompatibility for the Rhc antigen ($N = 121$). We conducted bivariate analyses to assess a relationship between perinatal factors (e.g., titre and concentration of anti-c antibodies, direct antiglobulin test) and HDFN, its severity and duration.

Results: The incidence of HDFN was 30% ($n = 36$), including 11% of severe HDFN ($n = 13$). Seven percent ($n = 9$) of neonates received at least one transfusion during the first week and 21% ($n = 26$) after this period until 3 weeks of life. During pregnancy, a concentration ≥7.5 IU/ml and a titre ≥4 and above were associated with HDFN and severe HDFN ($p < 0.05$). At birth, the high intensity of the quantitative direct antiglobulin test was associated with HDFN and severe HDFN ($p < 0.05$). A concentration ≥15 IU/ml is the best factor (area under curve [AUC] = 0.78) in predicting HDFN, followed by a titre ≥8 (AUC = 0.76).

Conclusion: Anti-c alloimmunization causes neonatal anaemia, which is often belated. Paediatricians have to be aware of these risk factors and organize prolonged monitoring of neonates.

KEYWORDS
anti-c alloimmunization, foetal and neonatal anaemia, haemolytic disease, neonatal jaundice, neonatal transfusion

Highlights

- Among fetuses and neonates with isolated anti-c maternal-fetal incompatibility and a positive DAT at birth, HDFN occurred in 30% of cases and severe HDFN requiring fetal or

neonatal transfusion, exchange transfusion or intravenous immunoglobulin in the first 7 days of life occurred in 11% of cases.

- In case of isolated anti-c maternal-fetal incompatibility, a titre ≥ 4 and/or a concentration of anti-c anibodies ≥ 7.5 IU/mL during pregnancy were both significantly associated with HDFN.
- In case of isolated anti-c maternal-fetal incompatibility, neonatal anemia can be belated with 21% of the neonates requiring blood transfusion after the first week of age. This result emphasizes the need for a prolonged follow-up of neonates after hospital discharge.

INTRODUCTION

With the introduction of Rh immune globulin in the majority of developed countries, the incidence of alloimmunization caused by anti-D (RH1) has decreased. In consequence, the proportion of alloimmunization caused by other antibodies, such as anti-Kell (KEL), anti-c (RH4) and anti-E (RH3), has relatively increased representing up to 78% of the alloimmunized pregnancies [1–5]. The prenatal management of these other alloimmunizations has been derived from the more largely described management of anti-D (RH1) immunization. However, these other allo-immunizations present differences that may have a significant impact on the expression and severity of the associated haemolytic disease of the foetus and newborn (HDFN) [4, 6]. Moreover, the natural history of anti-c alloimmunization in neonates may differ from what is observed in cases with anti-D alloimmunization [7, 8].

We studied a consecutive series of 121 cases of pregnancies with anti-c alloimmunization and maternal-foetal incompatibility with the primary objective to identify perinatal factors associated with HDFN. Our secondary objective was to identify perinatal factors associated with severe HDFN, and with neonatal transfusion after the first week of life.

MATERIALS AND METHODS

Patients and data collection

A retrospective study was conducted in the French National Reference Centre in Perinatal Hemobiology (CNRHP). The CNRHP is organized around two functional units working in close collaboration in Paris to monitor and manage immunized women and their children: a biological unit expert in perinatal immuno-haematology, and an obstetric-paediatric clinical unit. As part of its mission defined by the French Ministry of Health, the CNRHP collects all prenatal and neonatal data [9].

We extracted from our database the records of children born between 1 January 2013 and 31 December 2015, in Paris and the surrounding area, with a mother diagnosed with anti-c alloimmunization. We included all children presenting isolated Rhc maternal-foetal incompatibility proven by Rh-Kell blood phenotyping. We excluded children with the compatible phenotype (Rhc negative) or with elution test positive to another associated antibody.

The data collection was carried out by an obstetrician and the clinical paediatrician referent from the CNRHP. We first collected the biological samples including maternal screening for red blood cells antibodies and all available sera during pregnancy, direct antiglobulin test (DAT) and elution tests of the newborn. Medical files were reviewed to collect maternal characteristics, ultrasound follow-up and perinatal management of the cases. We also collected data from the delivery and the neonatal characteristics (haemoglobin and bilirubin, treatment of HDFN).

Prenatal monitoring

Rhc foetal genotyping was not performed in our center during the study period since it was implemented in 2015 [10]. Thus, if the fathers were cc homozygous, Rhc maternal-foetal incompatibility was certain; in case of Cc heterozygous fathers, pregnancies were followed as if they were incompatible, until otherwise proven at birth.

Anti-c antibody levels were quantified using two techniques: titration and concentration. The antibody concentration is an automated, continuous flow hemagglutination technique that is more accurate and demonstrates reactivation of alloimmunization more rapidly than titration [11]. In our center, the threshold for risk of foetal anaemia related to the anti-c antibody is defined by a concentration greater than 500 UCHP/ml (*units of the CNRHP*) [12]. This threshold of 500 UCHP/ml is comparable to the critical threshold of 7.5 IU/ml found in the UK Guidelines [13, 14]. It was considered that beyond 1000 UCHP/ml (i.e., approximately 15 IU/ml), the risk of HDFN was major. The threshold value used for the titration was 4 using the indirect antiglobulin test with the tube method (saline medium). Reactivation of the immunization was considered if the concentration increased more than 30% compared to the previous value or if the titration increased by more than two dilutions compared to the previous value [11].

The titration and the concentration were performed every 2–4 weeks from 18 weeks of gestation (WG) and every 2 weeks from 28 WG [14].

Ultrasonography and Doppler assessment of the Middle Cerebral Artery Peak Systolic Velocity (MCA-PSV) measurements were

performed for women having an anti-c concentration over 7.5 IU/ml, once a week until delivery. In case of MCA-PSV value increasing above 1.5 Multiples of Median (MoM), associated or not with indirect signs of foetal anaemia, a cordocentesis was performed to confirm anaemia, and perform an intrauterine transfusion (IUT) if necessary [15].

Severely anaemic foetuses were transfused with group O adult donor packed red cells, cross-matched with the mother, irradiated, fresh (<5 days) and with haematocrit at 70%–80%; Rhc negative (RH: 2,−4) and if possible, with respecting the extensive mother's phenotype (D, C, E, K, Jk, MNS, Fy).

For all patients with anti-c concentration ≥7.5 IU/ml, delivery at 37 weeks was recommended.

Postnatal monitoring

At birth, haemoglobin (Hb) level and bilirubin levels were measured on cord blood. An immunological diagnosis of incompatibility was performed including Rh-K blood phenotyping and quantitative DAT to test for the presence of anti-red blood cells bound antibodies (DAT performed on Bio-Rad Diaclon ABO/Rh for Newborns gels cards and

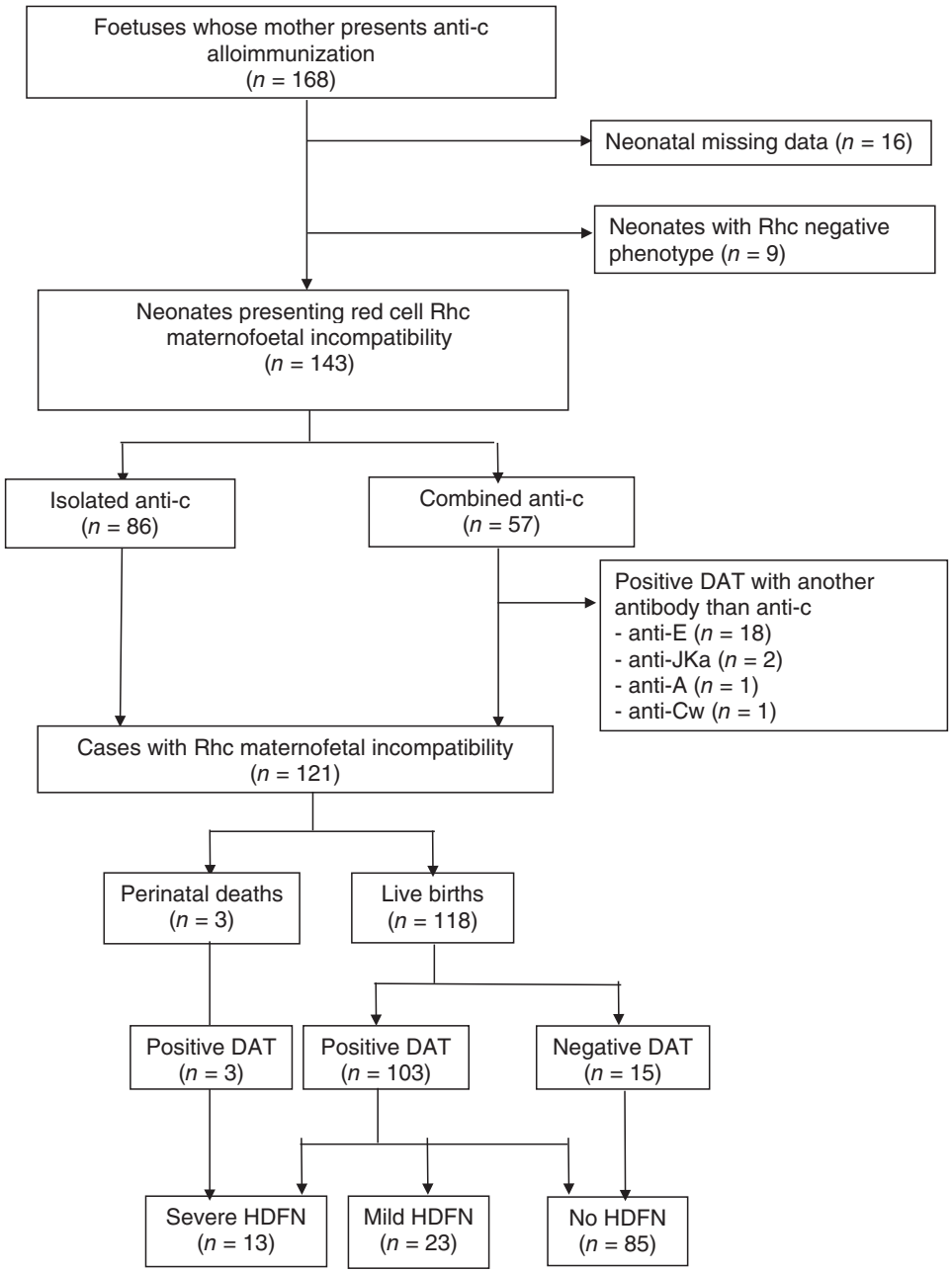


FIGURE 1 Flow-chart. DAT, direct antiglobulin test; HDFN, haemolytic disease of the foetus and newborn, defined as a neonatal anaemia with Hb less than 14 g/dl in the first hour of life and/or newborn pathologic icterus with adequate phototherapy based on bilirubinemia according to the clinical practice guidelines of the American academy of Pediatrics; severe HDFN, foetal or neonatal transfusion, exchange transfusion or intravenous immunoglobulin within the first 7 days of life

TABLE 1 Maternal and neonatal characteristics of the population ($n = 121$)

Maternal characteristics	
Maternal age (years)[a]	34 [30–37]
Gestity[a]	3 [2–4]
Parity[a]	2 [1–3]
History of HDFN[b]	
None	100 (83%)
Mild HDFN	10 (8%)
Severe HDFN	11 (9%)
Immunohaematologic characteristics	
Date of immunization[b]	
Before pregnancy	52 (43%)
First trimester	16 (13%)
Second trimester	17 (14%)
Third trimester	34 (28%)
Missing data	2 (2%)
Titre at delivery[b]	
<4	58 (47%)
4	9 (7%)
8	9 (7%)
>16	16 (13%)
Missing data	29 (27%)
Concentration at delivery (IU/ml)[a]	
<7.5	67 (55%)
≥7.5	28 (23%)
Missing data	26 (22%)
Reactivation of alloimmunization[b]	36/70 (51%)
Obstetrical characteristics	
Foetal transfusion[b]	2 (1%)
Gestational age at delivery[a]	38 [37–39]
Neonatal characteristics	
Birth weight (g)[a]	3150 [2892–3448]
Perinatal death[b]	3 (2%)
Direct antiglobulin test[b]	
0	15 (12%)
1+	24 (20%)
2+	19 (16%)
3+	24 (20%)
4+	39 (32%)
Haemoglobin at day 0 (g/dl)	15.3 [14.4–17.0]
Total bilirubin at day 0 (μmol/L)	38 [31–59]
Intensive phototherapy[b]	24 (20)
Blood transfusion <72 h	8 (7)
Exchange transfusion <72 h	3 (2)
Blood transfusion <7 days	9 (7)
Intravenous immunoglobulins	4 (3)
After 7 days	
Blood transfusion >7 days[a]	26 (21)
Age of the last transfusion (weeks)	3 [3–4.75]
Haemoglobin nadir (g/dl)	8.6 [7.6–10.2]

Abbreviation: HDFN, haemolytic disease of the foetus and newborn.
[a]Data are expressed in median [interquartile range].
[b]Data are *n* (%). Direct antiglobulin test was also measured on foetal blood sampling for the two cases with intrauterine transfusion.

on Bio-Rad DC Screening II gel cards using a 4-crosses scale to rate the intensity of hemagglutination). In case of DAT positivity, an elution test was realized to confirm the anti-c specificity of the antibodies. Iterative blood tests were repeated at 6 h and every 12 h during the first 72 h. HDFN was defined by neonatal anaemia with Hb less than 14 g/dl in the first hours of life and/or newborn hyperbilirubinemia requiring intensive phototherapy according to the clinical practice guidelines of the American Academy of Pediatrics [16]. Severe HDFN was defined by the need for foetal or neonatal transfusion, exchange transfusion or intravenous immunoglobulin within the first 7 days of life. Blood transfusions performed after the 7th day of life were also recorded.

Neonatal management of jaundice relied on intensive phototherapy and exchange transfusion when indicated in accordance with the American Academy of Pediatrics guidelines [16]. Intensive phototherapy was prescribed to maintain bilirubin levels below 340 μmol/L and prevent kernicterus as long as needed. Postnatal transfusion was recommended for Hb less than 8 g/dl and according to clinical tolerance [17]. The transfused blood was 25–30 ml/kg of group O packed red cells cross-matched with the newborn sera, irradiated (if previous foetal transfusions had been performed). A polyvalent immunoglobulin infusion was used to inhibit hemolysis if the bilirubin level increased from 8 to 10 μmol/h under intensive phototherapy. Some children were eligible for a combination of several treatments.

Clinical examination and blood tests (Hb level and reticulocyte count) were repeated within the first 2 months of life.

Ethics

Subjects have given their informed consent and the study protocol has been approved by the national committee in obstetrics and gynaecology research (CEROG 2020-OBS-0407).

Statistical analysis

We conducted bivariate analyses to assess a possible relationship between perinatal factors and HDFN, its severity and duration. Significant effects were based on the Chi-square (χ^2) test to compare categorical variables and Fisher's exact test was used when the assumptions of the χ^2 test were violated. Continuous variables were compared with the

TABLE 2 Risk factors of HDFN: Bivariate analysis

	No HDFN ($n = 85$), n (%)	HDFN ($n = 36$), n (%)	OR [95% CI]	p
History of HDFN	11 (13)	10 (28)	2.56 [0.87–7.56]	0.07
History of severe HDFN	6 (7)	5 (14)	2.11 [0.47–9.0]	0.30
Titre at first trimester				
≥4	3 (5)	7 (37)	9.69 [1.88–66.80]	0.002
≥8	2 (4)	7 (37)	14.7 [2.41–161.8]	<0.001
≥16	1 (2)	6 (32)	23.5 [2.54–1158]	<0.001
Titre during pregnancy				
≥4	14 (16)	21 (58)	7.1 [2.68–15.8]	<0.001
≥8	10 (12)	21 (58)	10.5 [4.12–26.8]	<0.001
≥16	5 (6)	14 (38)	10.2 [3.3–31.3]	<0.001
Concentration at first trimester				
≥7.5 IU/ml	6 (10)	8 (33)	4.33 [1.31–14.3]	0.04
≥15 IU/ml	4 (7)	4 (17)	2.66 [0.45–15.8]	0.22
Concentration during second or third trimester				
≥7.5 IU/ml	21 (25)	28 (78)	10.7 [4.2–27.0]	<0.001
≥15 IU/ml	6 (7)	19 (53)	14.7 [5.1–42.3]	<0.001
Date of immunization				
Second or third trimester	17 (31)	4 (23)	0.69 [0.14–2.69]	0.76
Discovery at the time of delivery	15 (18)	4 (11)	0.49 [0.08–1.92]	0.57
Reactivation at third trimester	23 (44)	13 (72)	3.3 [1.0–10.5]	0.05
Direct antiglobulin test				
0	15 (18)	0 (0)		
1+	21 (25)	3 (8)		
2+	17 (20)	2 (5)		<0.001
3+	15 (18)	9 (25)		
4+	17 (20)	22 (61)		

Abbreviations: CI, confidence interval; HDFN, haemolytic disease of the foetus and newborn; OR, odds ratio.

Wilcoxon rank-sum test. The odds ratios and the corresponding 95% confidence intervals were derived using bivariate analysis. Receiver operating characteristic (ROC) curves were performed to assess the performance of prediction of HDFN according to different thresholds of titration and concentration of anti-c antibodies during pregnancy. Statistical analyses were performed with R software version 3.5.1. *p Values* were used to estimate the strength of association with each parameter and statistical significance was set at $p < 0.05$.

RESULTS

Population

During the study period, we identified 168 pregnancies with anti-c alloimmunization referred to the CNRHP from 74 maternity hospitals. Among these pregnancies, 25 cases were excluded due to missing neonatal data in 16 cases or exclusion of maternal-foetal Rhc incompatibility (newborn with an Rhc negative phenotype) in 9 cases.

In our population of 143 pregnancies with anti-c alloimmunization and maternal-foetal incompatibility, 57 women had multiple red blood cell antibodies (40%). Among these cases of multiple immunizations, 22 were subsequently excluded because of a positive elution test to another antibody than anti-c at birth (Figure 1).

A total of 121 pregnancies fulfilled the inclusion criteria with neonates presenting isolated maternal-foetal incompatibility related exclusively to anti-c alloimmunization (Table 1).

Prenatal outcomes

Severe foetal anaemia occurred in three cases. In the first case, an IUT was performed at 21 WG for MCA-PSV over 1.5 MoM without hydrops (maternal titre was 512 and foetal Hb was 5.0 g/dl). Intrauterine foetal death (IUFD) was diagnosed the next day, due to a hematoma of the umbilical cord.

In the second case, IUFD occurred at 37 WG in a woman with an anti-c alloimmunization without prior appropriate follow-up. When a

TABLE 3 Risk factors of severe HDFN: Bivariate analysis

	No HDFN or not severe ($n = 108$), *n* (%)	Severe HDFN ($n = 13$), *n* (%)	OR [95% CI]	*p*
History of HDFN	17 (16)	4 (31)	2.36 [0.48–9.73]	0.24
History of severe HDFN	8 (6)	3 (18)	3.7 [0.54–18.9]	0.10
Titre at first trimester				
≥4	6 (10)	4 (36)	5.25 [0.87–29.55]	0.04
≥8	5 (8)	4 (36)	6.4 [1.0–38.5]	0.02
≥16	3 (5)	4 (36)	10.8 [1.5–90.0]	0.008
Titre during pregnancy				
≥4	27 (25)	8 (62)	4.80 [1.45–15.92]	0.01
≥8	20 (18)	11 (85)	24.2 [5.0–118]	<0.001
≥16	12 (11)	7 (54)	9.3 [2.7–32.4]	<0.001
Concentration at first trimester				
≥7.5 IU/ml	10 (14)	4 (40)	4.0 [0.71–20.9]	0.06
≥15 IU/ml	5 (7)	3 (30)	5.5 [0.71–36.7]	0.14
Concentration during pregnancy				
≥7.5 IU/ml	37 (34)	12 (92)	23.0 [2.9–184]	0.003
≥15 IU/ml	13 (12)	12 (92)	87.7 [10.5–731]	<0.001
Date of immunization				
Second or third trimester	19 (30)	2 (18)	0.52 [0.05–2.86]	0.72
Discovery at the time of delivery	17 (16)	2 (15)	0.95 [0.1–5.0]	1
Reactivation at third trimester	31 (49)	5 (71)	2.6 [0.46–14.3]	0.47
Direct antiglobulin test				
0	15 (14)	0 (0)		
1+	23 (21)	1 (8)		
2+	19 (18)	0 (0)		0.01
3+	22 (20)	2 (15)		
4+	29 (27)	10 (77)		

Abbreviations: CI, confidence interval; HDFN, haemolytic disease of the foetus and newborn; OR, odds ratio.

blood sample was performed at 35 WG for maternal purposes, the titre was 8 and the concentration was 38.1 IU/ml. Unfortunately, the ultrasound examination led to the diagnosis of an IUFD associated with foetal hydrops.

In the third case, a first IUT was indicated at 25 WG (maternal titre at 128, foetal haemoglobin raised from 5.2 to 11.2 g/L). A second IUT was planned 13 days later but secured cordonal access was impossible to achieve (maternal body mass index = 50 kg/m^2) and only a foetal blood sample was available with Hb level at 5.8 g/dl. The mother had a caesarean section 1 h later for a sinusoidal foetal heart rate at 26 WG. Palliative care was decided at 3 weeks of life because of a grade IV intraventricular haemorrhage and ischemic involvement of the basal ganglia leading to death at day 50.

Neonatal outcomes

Among the 118 live neonates, 103 (87%) had a positive DAT with anti-c at elution. A negative DAT was observed at birth in 15 neonates (absence of placental transfer of the anti-c antibodies to the newborns incompatible with their mother).

Twenty-four neonates (20%) required intensive phototherapy and four (3%) were treated with intravenous immunoglobulins. An exchange transfusion was required in three neonates (3%) with a high bilirubin level (358, 255 and 445 μmol/L, respectively) and a low haemoglobin level (6, 8.8 and 8.3 g/dl, respectively). A top-up transfusion was required in 29 neonates (24%), with a median of 2 transfusions (range: 1–3 transfusions) and the last transfusion at 3 weeks of age (range: 2–11 weeks). Nine neonates received at least one transfusion during the first week of age and 26 neonates (21%) required blood transfusion after this period. Among those, it was the first transfusion for 20 (17%) of them (Table 1).

Risk factors for HDFN

The incidence of HDFN was 30% ($n = 36$), including 11% of severe HDFN ($n = 13$). A titre ≥4 and above, a concentration ≥7.5 IU/ml during

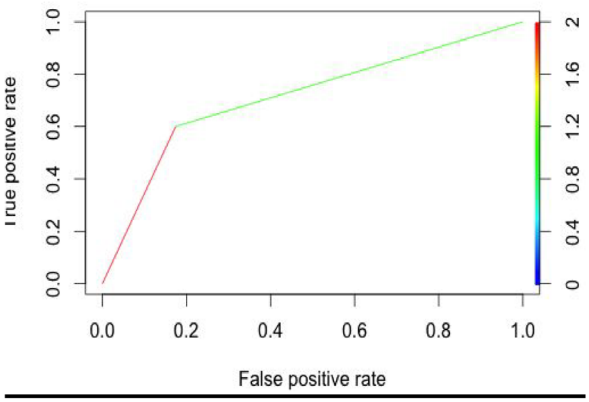
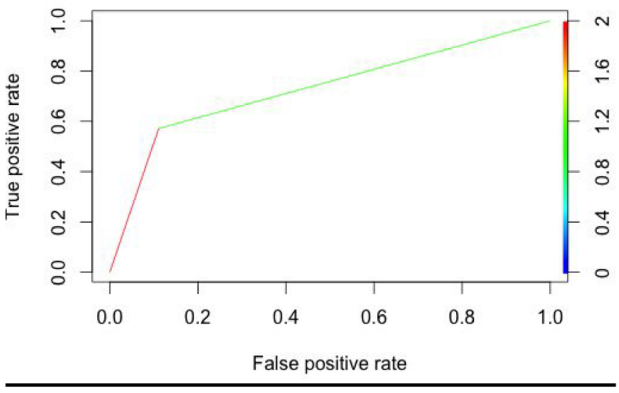
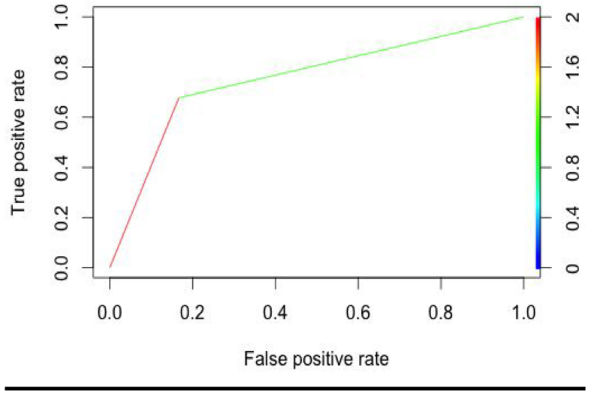
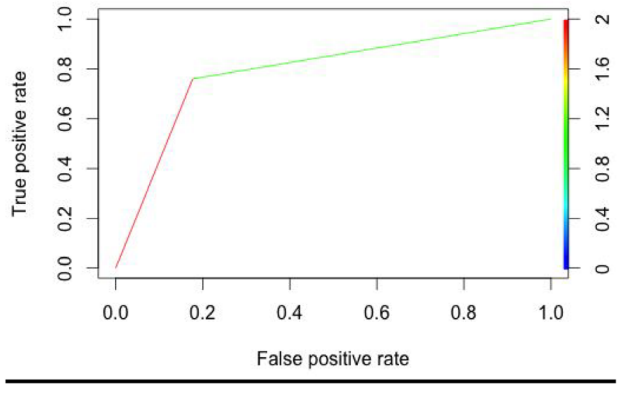
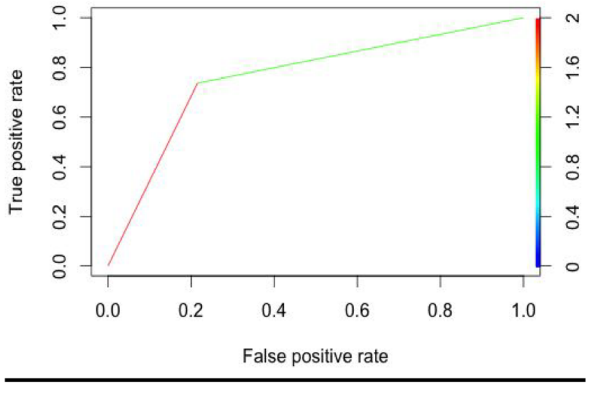
Concentration (IU/ml)	Titration
	≥4: area under curve = 0.71
	
≥7.5 IU/ml: area under curve = 0.73	≥8: area under curve = 0.76
	
≥15 IU/ml: area under curve = 0.78	≥16: area under curve = 0.76
	

FIGURE 2 Receiver operating characteristic curves for the performance of prediction of haemolytic disease of the foetus and newborn according to different thresholds of titration and concentration of anti-c antibodies during pregnancy

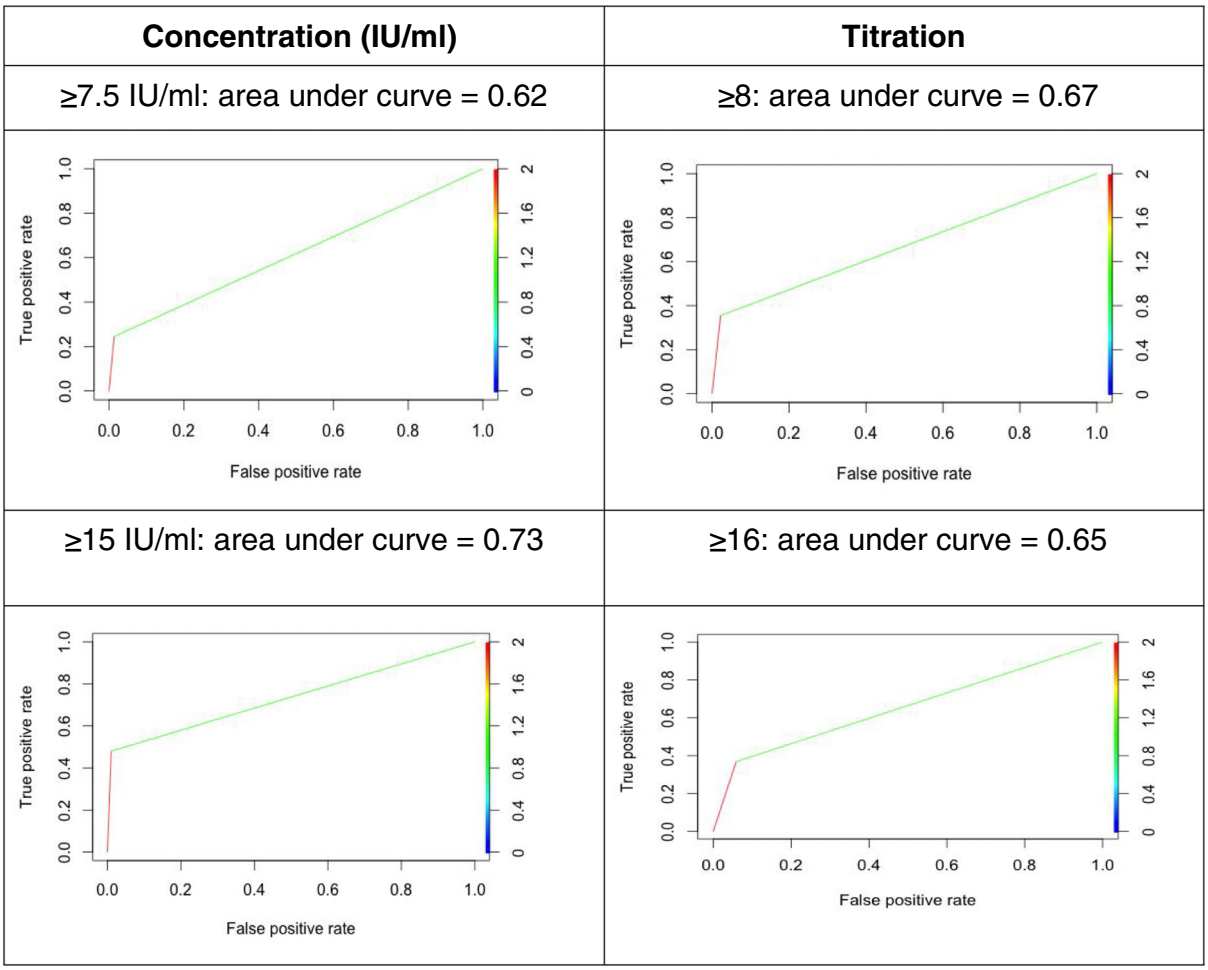


FIGURE 3 Receiver operating characteristic curves for the performance of prediction of severe haemolytic disease of the foetus and newborn according to different thresholds of titration and concentration during pregnancy

pregnancy and high intensity of the DAT were significantly associated with HDFN (Table 2). No association was found with a history of severe HDFN in a previous pregnancy, a concentration ≥15 IU/ml during the first trimester, the timing of immunization, and reactivation at third trimester. A titre of anti-c antibodies ≥8, a concentration ≥7.5 IU/ml throughout pregnancy and the strength of the DAT were significantly associated with severe HDFN (Table 3). The same criteria and medical history of severe HDFN were associated with a higher risk for the need of a postnatal transfusion at more than 7 days of life (Table S1). Using ROC curves, the concentration threshold of 15 IU/ml had the best performance (area under curve [AUC] = 0.78) in predicting HDFN, followed by a titre threshold of 8 or 16 (AUC = 0.76 for both) (Figure 2). Similarly, these three thresholds predicted severe HDFN with respective AUC of 0.73, 0.67 and 0.65 (Figure 3).

DISCUSSION

Among 121 foetuses and neonates with isolated anti-c maternal-foetal incompatibility, 106 (88%) had a positive DAT at birth. Among these 106 foetuses and neonates, HDFN occurred in 30% of cases and severe HDFN requiring foetal or neonatal transfusion, exchange transfusion or intravenous immunoglobulin in the first 7 days of life occurred in 11% of cases.

To the best of our knowledge, this is the largest series of pregnancies with anti-c alloimmunization for which perinatal care followed the most recent standard of care and current practice guidelines [12, 16–18]. Moreover, the strength of these results relies on the short inclusion period restricted to 2 years and the homogenous management of cases supervised by a single expert centre for perinatal haemobiology. The limits of our study are its retrospective design which leads to missing data and the limited number of severe cases. Data collection was exhaustive for the vast majority of criteria. Unfortunately, we did not present some criteria such as reticulocyte values due to missing data while they could have been of great interest.

Although several studies have shown that maternal anti-D titre is a good indicator of the severity of haemolytic disease, this had not been established in anti-c immunization due to the restricted number of cases in the previous series. This series allowed to determine thresholds helpful for all practitioners involved in the perinatal management of these

cases. Thus, a titre ≥4 and/or a concentration ≥7.5 IU/ml during pregnancy were both significantly associated with HDFN.

Considering our experience with anti-c quantitation, we found that a quantitative approach allows an earlier diagnosis of immune activation during pregnancy considering a longitudinal follow-up strategy of each case [11]. We thus observed that 500 UCHP/ml (CNRHP unit) could be brought back to 7.5 IU/ml in international unit and was more accurate than titration in the detection of reactivation (unpublished data).

The results of our study confirm our current policy of performing weekly MCA-PSV only if anti-c concentrations are ≥7.5 IU/ml. However, we must be careful with this result regarding foetal disease due to the use of a composite criterion including prenatal and postnatal outcomes, and the limited number of severe foetal cases.

Recently, Koelewijn et al. [19] used monocyte-driven antibody-dependent cellular cytotoxicity (ADCC) tests as a supplement to antibody concentration or titre to detect all cases at risk for severe HDFN. Repeated testing may add a more accurate risk estimation in cases with a titre ≥16. With an ADCC test ≥30%, a positive predictive value of 38% was obtained to detect severe HDFN.

Regarding the overall perinatal outcomes, we observed that anti-c immunization seems at low foetal risk. In this study, there were only 3 cases of severe foetal anaemia. The severe adverse outcomes we observed may be explained by initial very severe clinical cases or difficult technical conditions. These results are not representative of survival rates reported by our department and the other expert centers in the literature [20, 21]. In the majority of cases, in utero haemolytic risk is low, probably due to the low affinity of anti-c antibodies for foetal red blood cells or to other biochemical characteristics like the IgG-Fc glycosylation pattern of these antibodies [22]. Unlike our study, Rath et al. [6] report 41% of foetal transfusions in a small series of 22 foetuses with severe anti-c immunization. It is difficult to compare these results since in the Netherlands, only the most severe cases of anti-c immunizations with titres >16 are referred to the national reference center. Older series are not comparable since they included a majority of cases managed before implementation of non-invasive monitoring of MCA-PSV [23–25].

Like others, we have demonstrated that the neonatal risk of HDFN is high. The highest rate of top-up transfusion has been reported by Rath et al. close to 60% in a population of severe alloimmunization [6]. Unlike previous authors, our series allows to discriminate neonates at higher risk of HDFN and to offer appropriate postnatal management. At birth, quantitative DAT on cord blood appears as a very significant marker of the severity of HDFN. This very accessible test should be performed systematically as a postnatal marker to anticipate the risk of severe HDFN and organize appropriate follow-up. Thus, newborns with a negative DAT or a negative Rhc phenotype at birth do not have to be followed up for anti-c related HDFN. For others, follow-up can be weighted according to the strength of the DAT.

The history of HDFN in previous pregnancy does not appear to be associated with HDFN or severe HDFN. It can be explained by the low rate of patients with a history of HDFN (17%) in this population. We observed that 14% of the children of these patients had a transfusion or exchange transfusion in the first 72 h and half of them had a transfusion after 7 days regardless of the degree of history of HDFN. Paediatricians should be aware of this specific risk factor.

Finally, we observed a high rate of late top-up transfusions performed more than 7 days after birth (21%). This result emphasizes the need for a prolonged follow-up of neonates after hospital discharge.

Our national referral center currently uses Rhc foetal genotyping. For Rhc negative foetuses, the pregnancy continues to term doing antiglobulin testing monthly. For Rhc positive foetuses, we recommend inducing the delivery at 37 weeks if the alloimmunization is severe (concentration ≥ 7.5 IU/ml) and 39 weeks for moderate alloimmunization (concentration < 7.5 IU/ml). We also recommend performing the following cord blood samplings at birth: bilirubinemia, complete blood count including reticulocytes, ABO Group and Rhc antigen typing, DAT and elution. A paediatrician should examine the neonate looking for haemolytic disease. Bilirubin control at H6/H8, Rh Kell phenotype group determination and DAT must be performed on a peripheral blood sampling regardless of clinical manifestations. Treatment should be discussed according to biological results and evolution within the first 24 h. Monitoring of Hb level and reticulocyte count should be organized over the first 2 months of life if the DAT is positive regardless of the neonatal clinical picture [26].

In conclusion, perinatal factors predictive of HDFN in anti-c immunization comprise a titre ≥4 and a concentration ≥7.5 IU/ml during pregnancy and the strength of DAT positivity. The same criteria and a history of severe HDFN were associated with a higher risk of late postnatal transfusion beyond 7 days of life. Clinicians should be aware of these criteria to organize appropriate management of pregnancies complicated by anti-c immunization and need for referral in specialized tertiary centers.

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L.F., E.M. and J-M.J. conceptualized and designed the study and drafted the manuscript. C.T.N., S.H-J. and A.M. designed the data collection instruments, carried out the initial analyses and critically reviewed the manuscript. S.F. and P.M. carried out the final analyses and reviewed the manuscript. A.C. conceptualized and designed the study, supervised data collection and critically reviewed the manuscript.

CONFLICT OF INTEREST

The authors do not have any financial relationships and any conflicts of interest relevant to this article to disclose.

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REFERENCES

1. Urbaniak SJ, Greiss MA. RhD haemolytic disease of the fetus and the newborn. Blood Rev. 2000;14:44–61.
2. Moise KJ Jr. Non-anti-D antibodies in red-cell alloimmunization. Eur J Obstet Gynecol Reprod Biol. 2000;92:75–81.
3. Moise KJ. Red blood cell alloimmunization in pregnancy. Semin Hematol. 2005;42:169–78.
4. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Semin Fetal Neonatal Med. 2008;13:207–14.
5. Bowell PJ, Brown SE, Dike AE, Inskip MJ. The significance of anti-c alloimmunization in pregnancy. Br J Obstet Gynaecol. 1986;93:1044–8.
6. Rath ME, Smits-Wintjens VE, Lindenburg IT, Folman CC, Brand A, van Kamp IL, et al. Postnatal outcome in neonates with severe rhesus c compared to rhesus D hemolytic disease. Transfusion. 2013;53:1580–5.
7. Slootweg YM, Koelewijn JM, van Kamp IL, van der Bom JG, Oepkes D, de Haas M. Third trimester screening for alloimmunisation in Rhc-negative pregnant women: evaluation of the Dutch national screening programme. Br J Obstet Gynaecol. 2016;123:955–63.
8. Hackney DN, Knudtson EJ, Rossi KQ, Krugh D, O'Shaughnessy RW. Management of pregnancies complicated by anti-c isoimmunization. Obstet Gynecol. 2004;103:24–30.
9. Circulaire DHOS/SDO/DGS n° 2004-156 du 29 mars 2004 relative au Centre national de référence en hémobiologie périnatale. Available from: http://affairesjuridiques.aphp.fr/textes/circulaire-dhossdodgs-n-2004-156-du-29-mars-2004-relative-au-centre-national-de-reference-en-hemobiologie-perinatale/
10. Da Silva N, Deray M, Huguet-Jacquot S, Toly-Ndour C, Maisonneuve E, Oudin O, et al. Le génotypage foetal non invasif chez les femmes enceintes alloimmunisées. Transfus Clin Biol. 2017;24:312.
11. Toly-NDour C, Mourtada H, Huguet-Jacquot S, Maisonneuve E, Friszer S, Pernot F, et al. Clinical input of anti-D quantitation by continuous-flow analysis on autoanalyzer in the management of high-titer anti-D maternal alloimmunization. Transfusion. 2018;58:294–305.
12. Huguet-Jacquot S, Toly-Ndour C, Cortey A, Carbonne B, Mailloux A. Diagnostic et suivi biologiques des allo-immunisations anti-érythrocytaires chez la femme enceinte. Rev Francoph Lab. 2015;470:73–80.
13. White J, Qureshi H, Massey E, Needs M, Byrne G, Daniels G, et al. British Committee for Standards in haematology. Guideline for blood grouping and red cell antibody testing in pregnancy. Transfus Med. 2016;26:246–63.
14. Toly-NDour C, Huguet-Jacquot S, Delaby H, Maisonneuve E, Cortey A, Mailloux A. Quantification des anticorps anti-érythrocytaires chez la femme enceinte. Rev Biol Med. 2019;347:1–18.
15. Mari G, Deter RL, Carpenter RL, Rahman F, Zimmerman R, Moise KJ Jr, et al. Non invasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the blood velocity in anemic fetuses. N Engl J Med. 2000;342:9–14.
16. American academy of Pediatrics Subcomittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics. 2004;114:297–316.
17. Recommandations professionnelles de bonnes pratiques de la Haute autorité de santé. Transfusions de globules rouges homologues: produits, indications, alternatives; 2015. Haute Autorité de Santé. https://www.has-sante.fr/upload/docs/application/pdf/2015-02/transfusion_de_globules_rouges_homologues_-_produits_indications_alternatives_-_recommandations.pdf
18. Koelewijn JM, Vrijkotte TGM, Van Der Schoot CE, Bonsel GJ, De Haas M. Effect of screening for red cell antibodies, other than anti-D, to detect hemolytic disease of the fetus and newborn: a population study in The Netherlands. Transfusion. 2008;48:941–52.
19. Koelewijn JM, Slootweg YM, Folman C, van Kamp IL, Oepkes D, de Haas M. Diagnostic value of laboratory monitoring to predict severe hemolytic disease of the fetus and newborn in non-D and non-K alloimmunized pregnancies. Transfusion. 2020;60:391–9.
20. Friszer S, Maisonneuve E, Macé G, Castaigne V, Cortey A, Mailloux A, et al. Determination of optimal timing of serial in-utero transfusions in red-cell alloimmunization. Ultrasound Obstet Gynecol. 2015;46:600–5.
21. Zwiers C, Lindenburg ITM, Klumper FJ, De Haas M, Oepkes D, Van Kamp IL. Complications of intrauterine intravascular blood transfusion: lessons learned after 1678 procedures. Ultrasound Obstet Gynecol. 2017;50:180–6.
22. Sonneveld ME, Koelewijn J, de Haas M, Admiraal J, Plomp R, Koeleman CAM, et al. Antigen specificity determines anti-red blood cell IgG-fc alloantibody glycosylation and thereby severity of haemolytic disease of the fetus and newborn. Br J Haematol. 2017;176:651–60.
23. Kozlowski CL, Lee D, Shwe KH, Love EM. Quantification of anti-c in haemolytic disease of the newborn. Transfus Med. 1995;5:37–42.
24. Howard H, Martlew V, McFadyen I, Clarke C, Duguid J, Bromilow I, et al. Consequences for fetus and neonate of maternal red cell alloimmunization. Arch Dis Child Fetal Neonatal Ed. 1998;78:F62–6.
25. Astrup J, Kornstad L. Presence of anti-c in serum of 42 women giving birth to c-positive babies: serologic and clinical findings. Acta Obstet Gynecol Scand. 1977;56:185–8.
26. Mailloux A, Cortey A, Delatour V, Poupon C, Rota M, Schmitt F, et al. Analytical and clinical guidelines on neonatal bilirubinemia. Ann Biol Clin (Paris). 2020;78:383–97.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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