Irregular antibodies in pregnancy during the universal anti-RHD prophylaxis era: a survey of Spanish Hospitals

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Background - Although great advances have been made in the prevention of hemolytic disease of the fetus and newborn, especially for anti-D, irregular antibodies still appear. In order to ascertain the situation in Spain, we conducted a survey among hospitals treating pregnant women.

Materials and methods - We sent a survey to Spanish public hospitals comprising demographical data (ethnicity, number of pregnancies, type of fertilization), characteristics of antibodies (specificity, number, antigenic compatibility), cause of alloimmunization and the clinical consequences (fetal death, icterus, anemia) between 2016 and 2021. We characterized the risk posed by antibodies and their clinical impact according to previously described criteria.

Results - During the study period, 574,140 children were born in the responding hospitals. Antibodies were detected in 1,055 women with 1,112 pregnancies, resulting in a 0.19% alloimmunization prevalence. The most common antibodies were anti-D (No.=393, 28.7%), anti-c (No.=204, 14.9%), anti-E (No.=199, 14.6%), anti-M (No.=129, 9.4%), and anti-Kell (No.=114, 8.3%). Incorrect prophylaxis administration in the current or past pregnancy accounted for 88.6% of anti-D, and 12 (6.5%) arose from an RhD-positive transfusion. When analyzing incompatible pregnancies (No.=424; 38%), 103 (24.3%) presented severe or very severe hemolytic disease, most of which (No.=92, 89%) were produced by high-risk antibodies, while only 11 cases were caused by medium-risk antibodies.

Discussion - The prevalence of irregular antibodies during pregnancy in Spain is lower than previously described for other Western countries. The most important antibodies are anti-D, anti-c and anti-K. More efforts to characterize antibodies should be made in order to reduce their prevalence and clinical impact.

Keywords: *irregular antibodies, pregnancy, alloimmunization, hemolytic disease, prophylaxis.*

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INTRODUCTION

The discovery of blood groups and irregular antibodies (IAs) directed against red blood cell antigens made modern transfusion possible. IAs appear after sensitising events like transfusion, pregnancy, or organ transplantation; some can appear spontaneously. Patient factors[1,2] such as infection, inflammation, and transfusions or pregnancies carrying the foreign antigen, make patients more prone to alloimmunization. Individuals with rare blood phenotypes can lack antigens that are present in the blood donor pool, resulting in a higher rate of alloimmunization after transfusion.

IAs are the leading cause of hemolytic disease of the fetus and the newborn (HDFN)[3-5], which can result in life-threatening complications for the fetus[6]. The advent of intrauterine transfusion (IUT) and exchange transfusion (ET) transformed the prognosis for severely affected fetuses, reducing mortality and prematurity; nevertheless, fetal deaths still occur[7,8].

Anti-D antibodies can cause severe HDFN[9,10] and are the only IAs with an immunoglobulin-based prophylaxis[11]; however, despite being successfully implemented worldwide, some women still become sensitised[12,13], often without a recognisable sensitising event[14]. This lack of understanding precludes the implementation of further preventive measures. As RhD alloimmunization becomes scarcer[15], the role of other IAs becomes more evident[16].

There is scant evidence about the state of gestational alloimmunization in Spain. Studies from single centres by Solves in Valencia and Sanchez-Duran Barcelona[4,17] reported the IA prevalence over several years. Their data align with those of other Western countries; however, a global and current appraisal of the Spanish situation is lacking.

We aimed to ascertain the causes of alloimmunization among women in Spain, as well as the clinical impact of the IAs identified on fetal and neonatal wellbeing nationwide, to thus propose maternity care recommendations.

MATERIALS AND METHODS

Survey, data collection, and authorisation

We conducted a survey on gestational IAs between 2016 and 2021 throughout Spain. The study was approved by the Regional Ethics Committee (*Comité de Ética para la Investigación con Medicamentos de Cantabria, CEIM*), code 2021-065.

Regional Health Services provide maternal healthcare in Spain, through a network of local clinics and hospitals, involving midwives, obstetricians, and pediatricians. Eighty-five per cent of children are born in the 295 public hospitals, and the rest in private clinics.

Senior physicians were contacted in each region to engage with regional health authorities and participants at individual hospitals.

Hospital participants collected patients' demographic data and obstetric history (number of pregnancies, number of children, and the number of the pregnancy when the IA appeared). Questions included the specificity and titre of the IAs detected, paternal or fetal phenotype, the cause of alloimmunization (errors in RhD prophylaxis, previous sensitising events like abortion (either spontaneous or therapeutic) or obstetric complications, transfusion, transplantation, etc.), and the clinical manifestations and severity of HDFN in the fetus and neonate.

Clinical and laboratory data were extracted retrospectively from the laboratory information systems in each hospital. If records were incomplete, local researchers could contact the patient to gather missing clinical data.

Pregnancy management

Detection and management of alloimmunization is performed according to Guidelines of the Spanish Society of Obstetrics. An ABO RhD blood group and antibody screen are performed within the first trimester, before the 28th gestation week (GW), and after childbirth. RhD-negative women are given 300 µg of polyclonal Rho(D) Immune globulin (anti-RhD Ig, Igamad® 750, Grifols, Barcelona, Spain) at 28th GW, after sensitising events, and after giving birth to an RhD-positive child. This is the only anti-RhD available in Spain.

Pregnant women with IAs capable of causing HDFN are referred to a hospital for obstetric management. IUTs are performed at national reference hospitals following international guidelines[18,19]. Therapeutic options include pregnancy termination by caesarean section or induction of childbirth, ET, top-up transfusion, phototherapy, and life support measures.

Antibody classification and impact analysis

During the study period, all centres used gel card hemagglutination on automated platforms for the detection and identification of irregular antibodies by the indirect antiglobulin technique. All centres also employed

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Table I - *HDFN risk classification due to antibody specificity and severity classification according to clinical manifestations*

HDFN risk classification due to antibody specificity	**HDFN Risk**	**Antigen**
	High	D, Rh c, Kell
	Medium	E, other Rh or Kell antigens, Fy^a, Fy^b
	Low	ABO, Jk^a, Jk^b, MNSs and other MNS antigens, PP1Pk
	No risk	I, Lewis, P1, Lu, Yt, Other
HDFN severity classification	**Outcome**	**Definition**
	Very severe HDFN	• Uterine death • Intrauterine transfusions • Exchange transfusion after birth
	Severe HDFN	• Top-up transfusions • Preterm induction of labour
	Mild HDFN	• Without preterm induction of labour • Treatment with phototherapy suffices
	None	• No HDFN

HDFN: hemolytic disease of the fetus and newborn. Adapted from de Haas, 2015[18].

laboratory information systems for data management and archiving.

Identified IAs were stratified according to their relative HDFN risk following the proposal of de Haas 2015 (**Table I**). The clinical impact of IAs on pregnancies was evaluated using the same criteria[18] (**Table I**). Very severe HDFN corresponded to cases of intrauterine death, need for IUT and/or ET after birth. Severe HDFN included cases treated with top-up transfusions or those in which preterm induction of labour was needed. Mild disease was defined as cases without a need of preterm induction of labour and in whom treatment with phototherapy suffices.

To increase accuracy, the analysis of HDFN focused on cases where the maternal-fetal antigenic incompatibility was demonstrated by neonatal phenotyping. When a woman had two or more IAs, the analysis was conducted by grouping the specificities below the most harmful of them (i.e., a woman with anti-D, Fy^a, and anti-Le^a would be analyzed as "D combined"). See **Figure S1** in the *Online Supplementary Content*.

Data management and statistical analysis

Data were collected on an Excel spreadsheet (Microsoft, Redmond, WA, USA) and sent anonymized to the study coordinators. No personal information reached the coordinators. If, for any cause, some information could not be retrieved (for example, the cause of alloimmunization could not be found in the patients´ records), the item was classified as "not reported" and are shown in the different tables, for better understanding of the work and also to highlight the actual situation.

Analysis was performed using the SPSS program (IBM, Armonk, NY, USA).

RESULTS

Answering hospitals

Twenty-five (9%) hospitals out of the 275 public hospitals attending childbirths answered the survey[19]. These hospitals ranged from community hospitals to tertiary-level centres, and were located in thirteen geographical and socially different Regions (*Online Supplementary Content*, **Table SI**). During the study period, 2,215,000 children were born in Spain, of which 574,140 (26%) were born in the responding hospitals[20].

The hospitals that did not participate cited a lack of resources for research, data dispersion, or bureaucratic hurdles to accessing information.

Demographics

IAs were detected in 1,055 women with 1,112 pregnancies, resulting in an alloimmunization prevalence of 0.19%. Of these pregnancies 93% occurred naturally, while 5.8% resulted from fertility techniques and 1.2% was unknown. Median age was 34 years (range, 17-56), while 73% of women were 26-40 years old. Most mothers (72.8%) were Europeans, 10.2% Latin American, 6.3% came from Northern Africa and the Middle East, 4.4% from Sub-Saharan Africa, 2.9% were Hindustani, 1.1%

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Asian, and 2.3% had other origins. Twenty per cent of IAs were found during the first pregnancy. Demographic data are detailed in **Table II**.

Table II - *Demographic data*

Demographic	Data
Women (No.)	1,055
Pregnancies (No.)	1,112
Type of fecundation; No. (%)	
Natural	1,035 (93%)
IVF	64 (5.8%)
Unknown	13 (1.2%)
Mean age; median (range)	34; 34 (17-56)
Pregnancy age groups; No. (%)	
≤20	12 (1.1)
21-25	52 (4.7)
26-30	157 (14.1)
31-35	333 (29.9)
36-40	321(28.9)
41-50	80 (7.2)
> 50	2 (0.2)
Unknown	136 (13.9)
Ethnicity; No. (%)	
European	809 (72.8)
South American	113 (10.2)
Northern Africa and the Middle East	70 (6.3)
Sub-Saharan Africa	49 (4.4)
Hindustan	32 (2.9)
East Asia	12 (1.1)
Oceania / Pacific	1 (0.1)
Others	25 (2.2)
Unknown	1 (0.1)
Pregnancies with IAs per woman (No.; %)	1 (1,005; 95.3%)
	2 (46; 4.4%)
	≥3 (4; 0.4%)
	Unknown 3 (0.2%)
Pregnancy where the main IA is detected; No. (%)	
1st	226 (20%)
2nd	433 (39%)
3rd	207(19%)
4th or more	231(21%)
Unknown	15 (1%)

IA: irregular antibody IVF: *in vitro* fertilisation.

Antibodies detected

A total of 1,367 IAs were detected. Most pregnancies (No.=920, 82.7%) had only one antibody, while 192 (17.3%) had two or more. Antibody specificities are shown in **Table III**. The most common IAs were anti-D (No.=393, 28.7%), anti-c (No.=204, 14.9%), anti-E (No.=199, 14.6%), anti-M (No.=129, 9.4%), and anti-Kell (No.=114, 8.3%).

Table III - *Frequency and specificity of IAs detected in pregnancies (if several IAs are present in the same pregnancy, each specificity is counted as one)*

Specificity	No.	%
Total general	**1,367**	**100**
D	393	28.7
c	204	14.9
E	199	14.6
M	129	9.4
Kell	114	8.3
Jk^{a}	64	4.7
C	41	3.0
Cw	35	2.6
S	32	2.3
Le^{a}	29	2.1
Fy^{a}	23	1.7
G	19	1.4
Le^{b}	12	0.9
Lu^{a}	11	0.8
Jk^{b}	9	0.7
e	7	0.5
Kp^{a}	7	0.5
Lu^{b}	5	0.4
Fy^{b}	5	0.4
PP1PK	4	0.3
Wr^{a}	4	0.3
Jr^{a}	3	0.2
Di^{b}	3	0.2
M1	1	0.1
H	2	0.1
Yt^{a}	2	0.1
U	2	0.1
N	2	0.1
P1	2	0.1
In^{b}	1	0.1
CD36	1	0.1
k (cellano)	1	0.1
f (ce)	1	0.1

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Causes of alloimmunization

Causes of anti-D alloimmunization

We detected 393 cases of anti-D alloimmunization; however, the cause of alloimmunization was known in only 184 cases (46.8%) (**Table IV**). Among these, 163 (88.6%) derived from failure to correctly administer anti -D prophylaxis in the current or past pregnancy, while 12 (6.5%) arose from an RhD-positive red blood cell or platelet transfusion given for massive hemorrhage or hematological diseases. Only nine cases (4.9%) occurred during the pregnancy after correct anti-RhD Ig prophylaxis.

Causes for non-anti-D antibodies

The cause of anti-K, anti-c, and anti-E alloimmunization (**Table V**) was known in 30, 41, and 21% of cases, respectively. When the cause was known, IAs appeared after a sensitising event during pregnancy (47, 86, and 17% respectively), and the rest due to transfusion.

Clinical impact

We classified 57.9% of pregnancies as high risk of HFDN (**Table VI**).

Global clinical outcomes

Among the 1,112 pregnancies, the fetal and/or neonatal clinical course was reported in 79% of cases (No.=880). HDFN with any degree of severity was observed in 26.3% (No.=231) of those pregnancies, of which 40.3% were very severe (No.=93), 15.5% severe (No.=36), and 44.2% mild (No.=102).

HDFN in incompatible pregnancies (No.= 424)

When analysing the confirmed incompatible pregnancies (No.=424; 38%), 103 (24.3%) presented severe or very severe HDFN, most of which (No.=92, 89%) were produced by high-risk antibodies, while only 11 cases were caused by medium-risk IAs.

As predicted, most severe/very severe HDFN cases in incompatible pregnancies were caused by anti-D, anti-c,

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Table IV - *Causes of anti-D alloimmunization*

Causes of anti-D alloimmunization (No.=393)	No.	%
Alloimmunization without errors in prophylaxis	9	2.3
RhD-positive transfusion	12	3.1
Incorrect prophylaxis administration during the present gestation	79	23.2
Failure detected; moment not described	36	9.1
Anti-RhD Ig was not administered after a sensitising event (abortion, chorion biopsy, etc...)	32	8.1
Anti-RhD Ig was not administered at week 28	10	2.5
Anti-RhD Ig was not administered	1	0.2
Incorrect prophylaxis administration during a previous gestation	84	21.4
Anti-RhD Ig was not administered post-childbirth (72 h)	12	3.1
Previous sensitising event without evidence of anti-RhD Ig administration	72	18.3
Not reported	209	53.2
Total	393	100

No.= number of gestations; anti-RhD Ig: Rho(D) immune globulin.

Table V - *Causes of non-D alloimmunization (No.=717)*

Cause of alloimmunization	Anti-Kell		Anti-c		Anti-C		Anti-E		Other IAs	
	No.	%	No.	%	No.	%	No.	%	No.	%
Pregnancy-related sensitising event (*)	15	14%	51	35%	5	22%	20	13%	30	10%
Transfusion	17	16%	8	6%	-	-	12	8%	8	3%
Not reported	73	70%	85	59%	18	78%	117	79%	259	87%
Total	105	100%	144	100%	23	100%	149	100%	297	100%

Including all pregnancies, not just incompatible ones; (*)Abortion, amniocentesis, perinatal bleeding, chorion biopsy.

Table VI - *Pregnancies classified according to IAs found and their HDFN risk classification*

HDFN Risk due to the type of antibody detected	No.	%
High risk (D, Rh c, Kell)	644	57.9
Medium (E, other Rh / Kell antigens, Fy^a, Fy^b)	221	19.9
Low risk (ABO, Jk^a, Jk^b, MNSs and other MNS antigens, PP1Pk)	189	17.0
No risk (I, Lewis, P1, Lu, Yt, Other)	58	5.2
Total	1,112	100

HDFN: hemolytic disease of the foetus and newborn. Classified according to de Haas 2015[18]. No.: number of cases.

Table VII - *Correlation between the theoretical HDFN risk vs clinical manifestations*

Clinical manifestations	Theoretical antibody risk							
	High (No.=323; 76%)		Medium (No.=59; 14%)		Low (No.=27; 4%)		None (No.=6; 1.4%)	
	No.	%	No.	%	No.	%	No.	%
Anemia	52	16.1	5	9	-	-	-	-
Hydrops	19	5.9	1	2	-	-	-	-
Fetal death	4*	1.2	-	-	-	-	-	-
Loss of fetal wellbeing	1	0.3	-	-	-	-	-	-
Growth delay	3	0.9	1	1.7	-	-	-	-
Asymptomatic	244	75.5	52	88	27	100	6	1

*Antibody specificity: anti-D (two cases with high titre; 512 and 2,048), one anti-D (titre 256) plus anti-C (titre 1), and one anti-Fy^a (unknown titre).

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and anti-K alone or in combination with other antibodies. Anti-D alone or combined with other IAs accounted for nearly 73% (No.=58) of very severe and 42% (No.=10) of severe HDFN cases. This was also the case for mild HDFN cases, where 57.6% of cases were caused by anti-D.

Conversely, a high number of asymptomatic (No.=212, 50%) or mild HDFN (No.=92, 21.7%) cases were also seen in incompatible pregnancies.

Clinical manifestations

The most common clinical manifestations of HDFN throughout the series, regardless of compatibility status, were anemia (No.=57, 25.1%) and hydrops (No.=20, 7.9%). Four fetuses died due to high-risk IAs (two cases of anti-D, one anti-D plus anti-C, and one anti-Fy^a), while fetal growth delay or loss of fetal wellbeing was seen in three and one cases, respectively. Correlation between the theoretical HDFN risk and clinical manifestations can be seen in **Table VII**.

Fetal *versus* neonatal impact

We analyzed the correlation between the clinical impact of IAs on the fetus during pregnancy and neonatal health after delivery. Of the 65 fetuses with severe and very severe disease, 42 (65%) presented severe disease after childbirth, as expected. Additionally, 30 children (8.2%) among the 350 without evidence of HDFN during pregnancy developed very severe or severe HDFN after birth.

DISCUSSION

We have systematically analyzed the prevalence, causes of alloimmunization, and clinical impact of IAs identified during pregnancy in Spain, using a nationwide survey conducted in the era of universal maternal healthcare and routine Anti-RhD Ig prophylaxis. We believe our work accurately represents the situation of gestational alloimmunization and HDFN in Spain.

Alloimmunization and antibody frequency

The prevalence of IAs and their specificity during pregnancy vary between reports; nonetheless, all show the persistence of IAs against RhD and other common antigens, such as Rhc, RhE, Kell, or M. In our study, the prevalence of IAs during pregnancy (0.19%) is below that reported for other European countries, Canada, or the USA, where it ranges from 0.61% to 1.5%[13,21-28]. However, a recent

literature review found an overall lower (0.17%) prevalence of IAs, with some Western countries achieving an even lower prevalence[29]. This could be attributed to differences in study designs, but also to the different epidemiological contexts. Our study comprises more recent cases, at a time when women have fewer pregnancies and gestational care is improved, reducing the risk of alloimmunization. However, biases in data collection cannot be ruled out given the limited number of participating hospitals, resulting in a possibly underestimated prevalence.

Our IA distribution included anti-D (28.7%), anti-c (14.9%), anti-E (14.6%), anti-M (9.4%), and anti-Kell (8.3%) as the predominant IAs, with 58% of sensitized pregnancies at high risk of HFDN (**Table V**), representing 0.11% of all pregnancies. In a prevalence study at a single Spanish hospital, Solves detected that 0.63% of pregnancies managed at their hospital between 2011-2016 had clinically significant IAs. In that case, anti-D (24%), anti-E (18%), anti-c (11%), and anti-K (6.4%) were the most frequent IAs[17]. They did not delve on the causes of alloimmunization. Also in Spain, Sanchez-Duran[4] analyzed 337 pregnancies referred for alloimmunization between 2000-2017, reporting that 77% of them had clinically significant IAs, with 53% anti-D and 19% anti-K as the most prevalent. They did not infer the prevalence in their population or report the causes of alloimmunization.

In Western countries, the prevalence of anti-D has been reported to be between 8 and 64%[29], while the prevalence of non-RhD antibodies is also very variable, with anti-K reaching 20.9%, and anti-c 12% in different studies. Anti-E can be the most frequent IA, reaching 38.2%[26]. A recent bibliographic review also found that anti-D is the most common alloantibody (35.01%), followed by anti-K (14.69%), anti-E (11.21%), anti-c (5.48%), anti-C (4.93%), and anti-M (2.35%) worldwide[29].

While non-European women represent 13-15% of births in Spain[20], they comprised roughly 28% of IAs in our series, without significant differences in prevalent IAs (*data not shown*). This could result from suboptimal care during previous pregnancies in their native countries[30]. Gestational care is free to migrant women and would not be a concern once they are in Spain. Nevertheless, our study was not designed to assess this point.

Surprisingly, 21% of IAs were found during the first pregnancy. This could be explained partially by the presence of natural IAs, like anti-E (11%)[31] or anti-M (17%). Still, we also detected a high rate of anti-D (34%), anti-K (8%), and anti-c (6%), which usually appear after sensitising events. Unfortunately, in most cases (No.=186; 82%), this information was not available, or the sensitising cause could not be ascertained. We hypothesise that incomplete patients´ records or unreported events, like early abortions, could play a role.

When analysing pregnancies obtained through *in vitro* fertilisation, the IA distribution was similar to the other pregnancies (data not shown), which could be explained because many women had undergone repeated sensitising events during previous pregnancies and/or treatments.

Causes of immunization

The cause of RhD alloimmunization is of great interest because of its severe consequences and the possibility of prevention. Among our patients with anti-D, 43.8% of cases were due to errors in the administration of the prophylaxis protocol at some point during the current or previous pregnancy, and 3% were linked to incompatible transfusion. This leaves 53% with no documented cause (**Table IV**). These cases could be explained by missing data or an incomplete investigation of the sensitising event when the IA was detected. Also, they could be due to the lack of recognition of the current gestation as the leading cause of alloimmunization. Thus, the lack of an evident sensitising event can be reported as unknown. This pattern is consistent with data from other researchers in similar healthcare systems, where anti-D alloimmunization could not be attributed to any prophylaxis error and was attributed to the gestation itself. Finally, we also hypothesise the existence of some sensitising subclinical events (abortions, small volume fetal-maternal hemorrhages)[30]. We consider access to adequate healthcare plays a limited role as gestational healthcare is free of charge and decentralized.

Noticeably, few studies report failures in the application of the prophylaxis protocol. The difference in anti-D prevalence among studies could also be the result of the different prophylaxis schemes implemented in each country, with different anti-D doses and administration policies. Similarly, the quantification of post-natal fetal-maternal hemorrhage has become less common, precluding in some cases the administration of required additional anti-RhD Ig prophylaxis doses. Nonetheless,

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Spain applies a "maximal" prevention strategy, with high anti-D doses at 28 GW and childbirth. Indeed, authors in several countries[9,16,23,32] could not identify any cause other than the present pregnancy and advocated or implemented increased or more frequent doses in the anti-D gamma globulin protocol.

It has been argued that early abortion (<12 GW) can be a sensitising event; however, a systematic review[15] found no firm evidence supporting RhD prophylaxis after early abortion. Other factors –e.g., caesarean section, perinatal death, advanced gestational age, postnatal bleeding (>1,000 mL) and manual placenta removal, often in combination– have also been identified as primary risk factors for anti-D immunization[12].

Regarding significant non-Rhesus IAs, sensitising events during pregnancy among our patients comprised 47% anti-K, 86% anti-c, and 17% anti-C, whereas incompatible transfusion comprised the rest (**Table V**).

This aligns with the findings of other authors[10,28], who concluded that RBC (red blood cell) transfusion was the most important independent risk factor, followed by parity, major surgery, and hematological disease, together with a prior male child and caesarean section. This was highlighted by other authors[2,22,33], notably in the Netherlands, where phenotyping for K to avoid alloimmunization through transfusion was recommended and implemented with successful results. Also in the Netherlands, Verduin[2] concluded that IUT could induce durable additional IAs.

Finally, the BEST group recently reviewed the causes of alloimmunization and discovered that sharing blood-soiled syringes was a possible cause of alloimmunization in drug abuse[30] apart from not receiving prophylaxis during pregnancy, transfusion and transplantation. The low prevalence of intravenous drug abuse among women in Spain[34], makes this hypothesis unlikely.

Clinical consequences of IAs

Even with adequate healthcare, HDFN can have lethal outcomes. In a recent bibliographical review, Rodrigues[29] pointed out that, worldwide, a mean of 36.84% of alloimmunized pregnant women had a baby with HDFN. Among our cases with available clinical information (No.=880), about 14% (No.=129) of pregnancies presented severe or very severe HDFN; this increased to 24% (No.=103) in antigenically incompatible pregnancies (No.=424). Fetal and neonatal deaths, although uncommon (0.36% of those pregnancies), also occurred.

Focusing only on incompatible pregnancies, anti-D, anti-c, and anti-K comprised 90% of all severe and very severe cases during the gestational and neonatal period. Conversely, many asymptomatic (50%) or mild HDFN (21.7%) cases were also seen in incompatible pregnancies. This could be associated with a low IA titre, different IgG isoforms, or fetal antigenic development, and should be the subject of future research. We found no correlation between *in-utero* and neonatal HDFN severity; however, the numbers were too small for statistical inference.

Our data on clinical impact resemble those of Solves[17], who reported that 67% of anti-D patients required HDFN treatment, and 7% of pregnancies with IAs needed IUT, most of which needed transfusion or ET after birth, and one child died. Sanchez-Duran[4] analyzed 337 pregnancies with IAs, with 77% considered at risk. The global rate of livebirths and survival was 87% even though 92 IUTs had to be performed in 45 fetuses (23.2% of those at risk, 87% with anti-D). In the high-risk group with incompatible fetuses, six miscarriages, six perinatal deaths, and seven labour inductions occurred.

Similar data have been reported in Europe. Gudlaugsson[9] reported that IUT was performed in 3.8% of pregnancies, while 26.5% of neonates were treated for HDFN with phototherapy (24.2%), ET (9.8%), and simple transfusion (5.3%). Ainley[35] detected no fetal or neonatal deaths, and only one IUT was needed among 154 women. Liu, in 2021[24], reported that 8% of affected pregnancies required IUT, especially when anti-D was present with other IAs. Vlachodimitropoulou[8], identified anti-D and anti-K titres as predictors of fetal anemia and the need for IUT early in pregnancy, aligning with Slootweg´s findings[14] for anti-K in the USA. Yu[36] reported a 1.7% HDFN incidence among livebirths over 15 years due to ABO (78.1%) and RhD (4.3%) IAs. Rahimi-Levene[27] reviewed pregnancies during 2011, where severe HDFN developed in 6.8% pregnancies with IAs. Seventeen fetuses and newborns (0.02% of births) required RBC transfusions. One fetus died of hydrops.

When specifically analysing anti-K antibodies, Kamphuis[37], in the Netherlands in 2008, demonstrated good clinical outcomes of routine screening for anti-Kell antibodies during pregnancy. Other authors[1,14,28] also found that monitoring and anticipating anti-K resulted in improved outcomes.

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Limitations

As a retrospective study, our work has some limitations. The use of automated analytical platforms and electronic patient records could minimize data loss, but we cannot not rule out biased reports or information losses.

In a sizable proportion of cases, the causative factors for IAs or the fetal compatibility status were not reported. This is particularly disturbing for RhD due to its potentially damaging effects and because it is one of the main focuses of our work. This could be due to loss of information, but also highlights that, often, no explanation can be found other than pregnancy itself, as Gudlaugson[9], Thorup[13], and McCauley[23] reported. Additionally, we suspect that in many middle- or low-risk IAs, compatibility with the fetus or child is not checked during pregnancy or childbirth, and information is lost or not reported. The same can be said for the lack of information regarding fetal or newborn health.

Data were unavailable from many hospitals due to several constraints. Although we analyzed a large number of pregnancies from hospitals in different regions, with different population and income levels, the reduced number of hospitals could result in a representation bias. Moreover, participating centers may differ systematically (tertiary hospitals with more complex cases, better case management), which could distort our results. Finally, our study reflects the situation in a developed Western country and should be applied cautiously in other contexts.

Table VIII - *Proposals for maternal healthcare improvement*

Problem	Improvement suggestions
Failure to administer the anti-Rho gamma globulin dose during pregnancy or childbirth	• Reduce the number of women receiving non-optimal healthcare due to socioeconomic reasons: improve coordination between social and health services • Software integration between healthcare levels • Digitally assisted treatment protocols • Implementation of electronic prescription and administration software
Improve laboratory performance	• Staff training: D variants, anti-G, phenotyping • Automation: gel cards, antibody titration, online results transmission
Improve transfusion performance	• Computer alert systems in transfusion • Reduce the number of women sensitized by transfusion: transfuse phenotype Rh (D, C, c, E, e) and K compatible RBCs for at-risk women
Clinical situations where it is unclear if anti-D is needed	• Research conditions that require RhD gamma globulin administration (i.e., abortion)
Others	• Engage donors from populations with different ethnic backgrounds • Consider new fertility situations (oocyte or sperm donation) as a cause of alloimmunization • Implement fetal genotyping in maternal plasma to deliver precise gestational care • Create a national database of gestational alloimmunization and hemolytic disease

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CONCLUSIONS

Irregular antibodies during pregnancy in Spain have a lower prevalence but a similar clinical spectrum of HDFN as in other Western countries. Maternal healthcare is uniform and adequate; however, dangerous IAs, such as anti-D, anti-c, and anti-K, still occur. Addressing the failures in anti-D prophylaxis that still exist[38] and providing higher or more frequent anti-RhD Ig prophylaxis doses could be essential to improve results. Transfusion of Rh- (D, C, c, E, e) and K-compatible RBC to girls and women of childbearing age should become a universal policy.

Information about fetal compatibility and the causes of alloimmunization is lacking. To improve maternal healthcare, all causes of IAs, antibody monitorization, the presence of the cognate antigen in the fetus or neonate, should be thoroughly investigated during pregnancy and childbirth, and their clinical outcomes reported. Furthermore, information systems should be improved to better understand the factors causing alloimmunization and address them effectively. The unification of clinical records, creation of national databases with compulsory reporting of alloimmunization cases, and the training of all staff related to pregnancy care, would also impact IA prevention. Given this, our suggestions for improvement are reported in **Table VIII**. Finally, prospective research on gestational antibodies and their clinical impact is still needed.

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During the study, a woman taking part was allegedly murdered by her partner, together with their child. Let

this be a memorial for all women suffering from gender violence.

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Authorship contribution

IRA and EMD conceived and designed the study. MRA collected, curated, analyzed data and designed the tables. IRA wrote the drafts. EMD acted as scientific supervisor. All the Authors collected data at their hospitals, read and approved each draft.

The Authors declare no conflicts of interest.

REFERENCES

1. Koelewijn JM, Slootweg YM, Folman C, Kamp IL, Oepkes D, 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(2): 391-399. doi: 10.1111/trf.15631.
2. Verduin EP, Brand A, van de Watering LMG, Claas FHJ, Oepkes D, Lopriore E, et al. Factors associated with persistence of red blood cell antibodies in woman after pregnancies complicated by fetal alloimmune haemolytic disease treated with intrauterine transfusions. Br J Haematol. 2015; 168(3): 443-451. doi: 10.1111/bjh.13130.
3. Liu S, Ajne G, Wikman A, Lindqvist C, Reilly M, Tiblad E. Management and clinical consequences of red blood cell antibodies in pregnancy: a population-based cohort study. Acta Obstet Gynecol Scand. 2021; 100(12): 2216-2225. doi: 10.1111/aogs.14261.
4. Sánchez-Durán MÁ, Higueras MT, Halajdian-Madrid C, Avilés García M, Bernabeu-García A, Maiz N, et al. Management and outcome of pregnancies in women with red cell isoimmunization: a 15-year observational study from a tertiary care university hospital. BMC Pregnancy Childbirth. 2019; 19(1): 356. doi: 10.1186/s12884-019-2525-y.
5. Erickson ML. Alloimmunization in pregnancy. In: *Immunologic concepts in transfusion medicine*. Amsterdam: Elsevier; 2020. p. 149-165. doi: 10.1016/B978-0-323-67509-3.00009-3.
6. Li S, He Z, Luo Y, Ji Y, Luo G, Fang Q, et al. Distribution of maternal red cell antibodies and the risk of severe alloimmune haemolytic disease of the foetus in a Chinese population: a cohort study on prenatal management. BMC Pregnancy Childbirth. 2020; 20(1): 539. doi: 10.1186/s12884-020-03235-w.
7. Franchinard L, Maisonneuve E, Friszer S, Toly Ndour C, Huguet-Jacquot S, Maurice P, et al. Perinatal risk factors associated with severity of haemolytic disease of the foetus and newborn due to Rhc maternal-foetal incompatibility: a retrospective cohort study. Vox Sang. 2022; 117(4): 570-579. doi: 10.1111/vox.13215.
8. Vlachodimitropoulou E, Garbowski M, Anne Solomon S, Abbasi N, Seaward G, Windrim R, et al. Outcome predictors for maternal red blood cell alloimmunisation with anti-K and anti-D managed with intrauterine blood transfusion Br J Haematol. 2022; 196(4): 1096-1104. doi: 10.1111/bjh.17956.
9. Gudlaugsson B, Hjartardottir H, Svansdottir G, Gudmundsdottir G, Kjartansson S, Jonsson T, et al. Rhesus D alloimmunization in pregnancy from 1996 to 2015 in Iceland: a nation-wide population study prior to routine antenatal anti-D prophylaxis. Transfusion. 2020; 60(1): 175-183. doi: 10.1111/trf.15635.
10. Koelewijn J, Vrijkotte T, de Haas M, van der Schoot C, Bonsel G. Risk factors for the presence of non-rhesus D red blood cell antibodies in pregnancy. BJOG. 2009; 116(5): 655-664. doi: 10.1111/j.1471-0528.2008.01984.x.
11. Lieberman L, Lopriore E, Baker JM, Bercovitz RS, Christensen RD, Crighton G, et al. International guidelines regarding the role of IVIG in the management of Rh- and ABO-mediated haemolytic disease of the newborn. Br J Haematol. 2022; 198(1): 183-195. doi: 10.1111/bjh.18170.
12. Slootweg YM, Zwiers C, Koelewijn JM, van der Schoot E, Oepkes D, van Kamp IL, et al. Risk factors for RhD immunisation in a high coverage prevention programme of antenatal and postnatal RhIg: a nationwide cohort study. BJOG. 2022; 129(10): 1721-1730. doi: 10.1111/1471-0528.17118.
13. Thorup E, Clausen FB, Brodersen T, Dellgren CD, Ekelund C, Haunstrup TM, et al. Evaluation of the clinical effect of a nationwide implementation of targeted routine antenatal anti-D prophylaxis in Denmark. Transfusion. 2025; 65(1): 29-37. doi: 10.1111/trf.18072.
14. Slootweg YM, Lindenburg IT, Koelewijn JM, Van Kamp IL, Oepkes D, De Haas M. Predicting anti-Kell-mediated hemolytic disease of the fetus and newborn: diagnostic accuracy of laboratory management. Am J Obstet Gynecol. 2018; 219(4): 393.e1-393.e8. doi: 10.1016/j.ajog.2018.07.020.
15. Chan MC, Gill RK, Kim CR. Rhesus isoimmunisation in unsensitised RhD-negative individuals seeking abortion at less than 12 weeks' gestation: a systematic review. BMJ Sex Reprod Health. 2022; 48(3): 163-168. doi: 10.1136/bmjsrh-2021-201225.
16. Sørensen K, Stjern HE, Karlsen BAG, Tomter G, Ystad I, Herud I, et al. Following targeted routine antenatal anti-D prophylaxis, almost half of the pregnant women had undetectable anti-D prophylaxis at delivery. Acta Obstet Gynecol Scand. 2022; 101(4): 431-440. doi: 10.1111/aogs.14328.
17. Solves P, Gomez-Seguí I, Osorio J, Martinez F, Perales A, Sanz MA, et al. Prevalence of red blood cell alloantibodies in pregnant women and hemolytic disease of newborn in a tertiary care hospital. ARC J Gynecol Obstet. 2017; 2(2): 18-23. doi: 10.20431/2456-0561.0202005.
18. de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Haemolytic disease of the fetus and newborn. Vox Sang. 2015; 109(2): 99-113. doi: 10.1111/vox.12265.
19. Ministerio de Sanidad. [Perinatal care in Spain. Analysis of human physical outcomes, activity and quality of hospital services 2010-2018]. Available at: https://www.sanidad.gob.es/en/biblioPublic/publicaciones.do?metodo=detallePublicacion&publicacion=27. Accessed on 31/07/2025. [in Spanish.]
20. Instituto Nacional de Estadística. [Main population series since 1998]. Available at: https://www.ine.es/jaxi/Datos.htm?path=/t20/e245/p08/l0/&file=01006.px. Accessed on 1/08/2023. [in Spanish.]
21. Erikstein BS, Hagen KG, Hervig T. RBC alloantibody prevalence and specificity in a Western Norwegian tertiary hospital. Transfus Med. 2019; 29(3): 169-178. doi: 10.1111/tme.12511.
22. Ainley L, Jardim JR, Tan J, Beski S, RiosLeal EM, Allard S, et al. Prevalence of maternal alloantibodies in a large teaching hospital and their impact on outcomes of fetuses/neonates. Transfus Med. 2017; 27(3): 228-230. doi: 10.1111/tme.12399.
23. McCauley CJ, Morris K, Maguire K. A review of maternal alloimmunisation to Rh D in Northern Ireland. Transfusion Medicine [Internet]. 2017; 27(2): 132-135. doi: https://doi.org/10.1111/tme.12387.
24. Liu S, Ajne G, Wikman A, Lindqvist C, Reilly M, Tiblad E. Management and clinical consequences of red blood cell antibodies in pregnancy: A population-based cohort study. Acta Obstet Gynecol Scand. 2021; 100(12): 2216-2225. doi: 10.1111/aogs.14261.
25. Sugrue RP, Moise KJ, Federspiel JJ, Abels E, Louie JZ, Chen Z, et al. Maternal red blood cell alloimmunization prevalence in the United States. Blood Adv. 2024; 8(16): 4311-4319. doi: 10.1182/bloodadvances.2023012241.
26. Moinuddin I, Fletcher C, Millward P. Prevalence and specificity of clinically significant red cell alloantibodies in pregnant women-a study from a tertiary care hospital in Southeast Michigan. J Blood Med. 2019; 10: 283-289. doi: 10.2147/JBM.S214118.
27. Rahimi-Levene N, Chezar J, Yahalom V, Abed-Alenbi A, Akira L, Arbov L, et al. Red blood cell alloimmunization prevalence and hemolytic disease of the fetus and newborn in Israel: a retrospective study. Transfusion. 2020; 60(11): 2684-2690. doi: 10.1111/trf.15987. Epub 2020 Aug 8. Erratum in: Transfusion. 2022; 62(7): 1466. doi: 10.1111/trf.16663.

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28. 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(5): 941-952. doi: 10.1111/j.1537-2995.2007.01625.x.
29. Rodrigues MMO, Mattos D, Almeida S, Fiegenbaum M. Hemolytic disease of the fetus and newborn-a perspective of immunohematology. Hematol Transfus Cell Ther. 2024; 46 Suppl 5(Suppl 5): S246-S257. doi: 10.1016/j.htct.2024.04.122.
30. Thorup E, Clausen FB, Brodersen T, Dellgren CD, Ekelund C, Haunstrup TM, et al. Evaluation of the clinical effect of a nationwide implementation of targeted routine antenatal anti-D prophylaxis in Denmark. Transfusion. 2025; 65(1): 29-37. doi: 10.1111/trf.18072.
31. Luken JS, Folman CC, Lukens MV, Meekers JH, Ligthart PC, Schonewille H, et al. Reduction of anti-K-mediated hemolytic disease of newborns after the introduction of a matched transfusion policy: a nation-wide policy change evaluation study in the Netherlands. Transfusion. 2021; 61(3): 713-721. doi: 10.1111/trf.16276.
32. Ainley L, Jardim JR, Tan J, Beski S, RiosLeal EM, Allard S, et al. Prevalence of maternal alloantibodies in a large teaching hospital and their impact on outcomes of fetuses/neonates. Transfus Med. 2017; 27(3): 228-230. doi: 10.1111/tme.12399.
33. Yu D, Ling LE, Krumme AA, Tjoa ML, Moise KJ. Live birth prevalence of hemolytic disease of the fetus and newborn in the United States from 1996 to 2010. AJOG Glob Rep. 2023; 3(2): 100203. doi: 10.1016/j.xagr.2023.100203.
34. Kamphuis MM, Lindenburg I, van Kamp IL, Meerman RH, Kanhai HHH, Oepkes D. Implementation of routine screening for Kell antibodies: does it improve perinatal survival? Transfusion. 2008; 48(5): 953-7. doi: 10.1111/j.1537-2995.2007.01626.x.
35. Contreras M, Kumpel B, Olovnikova N. Anti-D prophylaxis should protect all newborns from haemolytic disease, regardless of their country of residence. Vox Sang. 2024; 119(12): 1221-1222. doi: 10.1111/vox.13745.

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