ISBT Science Series
An affiliated publication to Vox Sanguinis

ISBT Science Series (2021) 0, 1–13

CONGRESS REVIEW

DOI: 10.1111/voxs.12617

Rh disease prevention: the European Perspective

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Background and Objectives In Europe, postnatal and subsequently antenatal administration of anti-RH1 (D) immunoglobulins (Ig) has reduced the number of maternal anti-RH1 immunizations and the incidence of haemolytic disease of the foetus and newborn since the 1960s. Non-invasive foetal *RHD* genotyping now enables antenatal prophylaxis to be targeted only to women carrying *RHD*-positive foetus. We aimed at describing how Rh disease prevention is currently managed in different European countries.

Materials and Methods We prepared an online survey on guidelines and biological tests performed for Rh disease prevention. The link was sent to 15 expert laboratories among Europe, selected by their publications in the field.

Results Experts from thirteen countries responded. Guidelines on anti-RH1 prophylaxis are similar regarding the major aspects of RH disease prevention, including indication and timing of anti-RH1 Ig administration, as well as indication of foetal *RHD* genotyping. Different anti-RH1 Ig preparations are used, and the dosing may differ depending on gestational age. Other controversial issues include (1) timing for foetal *RHD* genotyping, (2) indication of tests performed to quantitate feto-maternal haemorrhage prior to anti-RH1 Ig administration, (3) if there is a remaining indication for newborn RH1 phenotyping. Procedures for monitoring the prophylaxis efficiency and evaluating the national prevention programme also differ among countries.

Received: 4 December 2020, accepted 16 December 2020

Conclusion Despite some differences among countries, the Rh disease prevention policies are very efficient in Europe, but HDFN cases due to maternal anti-RH1 immunization have not completely disappeared. It therefore remains important to share best practices for continuous improvement in reducing anti-RH1 alloimmunization.

Key words: haemolytic disease of the foetus and newborn, foetal testing, alloimmunization, genotyping, immunoglobulins, serological testing.

Introduction

RH1 (RhD)-negative or variant RH1 women exposed to RH1-positive foetal cells during pregnancy may develop anti-RH1 (D) alloimmunization. The main causes of anti-RH1 sensitization during pregnancy are feto-maternal haemorrhage due to invasive gesture, trauma, antenatal spontaneous haemorrhage, abortion or delivery. Anti-RH1 alloimmunization can induce haemolytic disease of the foetus and newborn (HDFN) which can lead to foetal anaemia, hydrops fetalis, kernicterus and neonatal death [1].

Anti-RH1 immunoglobulin (Ig) administration to RH1-negative pregnant women had made HDFN caused by anti-RH1 alloimmunization a preventable disease. In the 1960s, the implementation of postpartum anti-RH1 Ig administration, followed by targeted antenatal anti-RH1 Ig administration in situation at risk of feto-maternal haemorrhage a few years later, allowed to decrease the anti-RH1 alloimmunization rate for RH1-negative women carrying a RH1-positive child from 15 to 1·6 % [2].

Despite the proven benefit of postpartum and targeted antenatal prophylaxis, sensitizations still occurred during the third trimester of pregnancy and were thought to be the result of small volume unprovoked feto-maternal haemorrhages. That is why in the 1990s routine immunoprophylaxis was secondarily introduced at the beginning of the third trimester, either by a single large dose of anti-RH1 Ig around 28 gestation weeks (GW) or by two smaller doses at 28 and 34GW [3]. Overall, the implementation of routine immunoprophylaxis further halved the incidence of anti-RH1 alloimmunization in European countries [4].

Since anti-RH1 Ig is derived from pooled donor plasma, there is theoretically a potential risk of transmission of blood-borne diseases. Moreover, some countries have problems in obtaining sufficient anti-RH1 Ig supplies, as these products are derived from special plasma, donated in a few countries for the whole world. The implementation of *RHD* foetal genotyping on maternal blood during the 2000s allowed the immunoprophylaxis to be given only to RH1-negative women carrying an *RHD*-positive or undetermined foetus, which represents 60% of cases.

To address the continuing challenges of Rh disease, a multidisciplinary international organization was founded in 2019: the Worldwide Initiative for Rh Disease Eradication (WiRhE). WIRhE aims to eradicate Rh disease by 'connecting the world to protect mothers and babies'.

In this context, we tried to provide an overview on how Rh disease prevention is currently managed in different European countries.

Material and methods

We prepared an online survey organized in 11 chapters and containing 56 questions to assess how Rh disease prevention is managed in European countries (see Appendix S1). The questions were about the existence of national guidelines, the type of anti-RH1 immunoglobulins used, foetal *RHD* genotyping, newborn RH1 phenotyping, diagnosis and quantification of feto-maternal haemorrhages, antepartum and postpartum immunoprophylaxis, assessment of the prevention efficacy, differentiation between passive and immune anti-RH1 in the maternal blood, follow-up of errors or omissions of the prophylaxis and overall impact of the prevention policy on anti-RH1 immunization cases.

An e-mail containing the link to the survey was sent twice the 20 January and the 5 February 2020 to local contacts of 15 European countries (Switzerland, Slovenia, Denmark, Italia, Luxemburg, Czech Republic, Finland, Ireland, Germany, Spain, United Kingdom, Belgium, Poland, Sweden, The Netherlands and France). The contact details of the laboratories were obtained after a query in the PubMed database, looking for publications in the field. The deadline to complete the survey was the 7 February 2020.

Results

Thirteen countries (Switzerland, Slovenia, Sweden, Denmark, Italy, Finland, Ireland, Germany, Spain, Belgium, Poland, The Netherlands and France) answered to the online survey. For Sweden (Regions Skåne and Stockholm, here denoted South and North, respectively) and Denmark, there were two answers coming from two different regions of the country.

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Twelve countries have national guidelines on Rh disease prevention (see Appendix S2).

Three main anti-RH1 Ig drugs are used with different types of dosing: Rhophylac® (CSL Behring, King of Prussia, PA, USA, 1000 and 1500 IU), Rhesonativ® (Octapharma, Lachen, Switzerland, 625 and 1250/1500 IU) and RhoGAM® (Kedrion S.p.A, Barga, Italy, 1500 IU).

The administration route is mainly intramuscular (IM), but some countries (Ireland, France) prefer the IV (intravenous) route.

The overall practice is similar with a 1250 to 1500 IU dosing at 28–30 GW for routine antenatal prophylaxis and a 1000 to 1500 IU dosing for targeted antenatal prophylaxis. Some countries (Denmark, Ireland and Slovenia) are using a lower dose (625 IU) for targeted prophylaxis at early gestational ages (Table 1). For post-delivery prevention, most of the countries use a 1250 to 1500 IU dosing, except the Netherlands, France (1000 IU) and Poland (750 IU).

Non-invasive foetal *RHD* genotyping is currently performed in the 13 countries that have responded. For 8 of them, the costs of the programme are fully covered by the government or by local institutions. This test is recommended at different gestational ages ranging from 10 to 27 GW. Three types of strategies are emerging as follows: (1) countries that recommend to perform the test as early as possible to adapt targeted antenatal prophylaxis for situations at risk of feto-maternal haemorrhage at early gestational age (Belgium, Sweden, Spain, Germany, Poland, Ireland and France), (2) countries that advocate this assay during the second trimester to increase its sensitivity (Italy and Switzerland) and (3) countries that advise to do it just before the third trimester to adapt routine antenatal prophylaxis (The Netherlands, Slovenia, Denmark and Finland).

To ensure a good sensitivity and avoid false-negative results, four countries are using foetal DNA markers and eight are systematically controlling negative results (Table 2).

Knowing the foetal *RHD* status is essential to guide maternal antenatal prophylaxis. But the approach at delivery differs between countries. Seven countries are systematically performing RH1 phenotyping on the newborn's blood whereas four are using the foetal *RHD* genotype to guide postpartum anti-RH1 Ig administration. In France, a newborn RH1 phenotype is performed only if the foetal *RHD* genotyping is undetermined or negative. In Slovenia, the RH1 phenotype is realized on the newborn's red blood cells only if the foetal *RHD* genotyping was found positive.

RH1 phenotyping is allowed on cord blood for Rh disease prevention in all the countries. In 9/13 countries, the reagent used must recognized the DVI partial antigen (Table 2).

Detection and quantification of feto-maternal haemorrhage (FMH) in a situation of a potentially sensitizing event are systematically performed in 7 countries. Among them, 6 are using the colorimetric Kleihauer–Betke test by microscopy and 5 are looking for the presence of circulating foetal red blood cells by flow cytometry.

Concerning the delivery, only Ireland, Slovenia, Belgium and France are systematically conducting a test to diagnose and quantitate FMH to adjust the anti-RH1 Ig dose to administer. Other countries (Switzerland, Sweden, Germany, Poland, Spain and Finland) are doing the test during pregnancy or at delivery only if a clinically significant FMH is suspected (Table 3).

In all countries, antepartum Rh immunoprophylaxis is systematically offered to partial RH1 pregnant women in situation at risk of FMH and for third trimester routine administration. The same procedure is applied for women with non-characterized *RHD* variant alleles. The administered anti-RH1 Ig doses are similar to the doses used for RH1-negative women. Comments were made by a number of participants about weak *RHD* type 1,2 and 3 that are considered as RH1 positive and the women carrying those variants do not receive anti-RH1 Ig (Table 4).

For postpartum Rh immunoprophylaxis, there is a time limit that must not be exceeded for anti-RH1 Ig administration in all participating countries. This time limit is 72 h in all but one country (48 h for the Netherlands).

If the time limit is exceeded, the situation is managed in different ways depending if the 'actions to be taken' are described or not in the national guidelines. For 5 countries, there is no detail in the recommendation concerning how long after delivery anti-RH1 Ig could be given. For the eight remaining countries, the proposed time limit varies between 1 and 4 weeks. If the newborn's phenotype is RH1 positive, immunoprophylaxis is offered to RH1-negative women but also to women carrying a partial RH1 antigen or a non-characterized *RHD* variant allele. As for antenatal prophylaxis, only women with weak *RHD* type 1,2 or 3 are not being given anti-RH1 Ig because of the proven lack of immunization risk [5] (Table 4).

The follow-up of Rh disease prevention efficacy is quite different among countries.

Concerning immediate efficacy after anti-RH1 Ig treatment, in case of initially positive Kleihauer test, only five countries check the test reversion after anti-RH1 Ig administration. Four countries make the recommendation to check for the presence of circulating anti-RH1 at the antibody screening after anti-RH1 Ig administration. To be sure of the anti-RH1 Ig treatment long-term efficacy,

Table 1 Anti-RH1 Ig Preparations, routes and dosing used

Country	Type of prophylaxis	Rhophylac® 200 µg (1000 IU) CSL Behring	Rhophylac® 300 µg (1500 IU) CSL Behring	RhoGAM® 300 µg (1500 IU) Kedrion S.p.A	Rhesonativ® 125 µg (625 IU) Octapharma	Rhesonativ® 250/300 µg (1250/1500 IU) Octapharma	Gamma anty-D® 150 µg (750 IU) Biomed Lublin
Switzerland	Targeted antenatal		X				
	Routine (28–32 GW)		X (IV)				
	Postpartum		X (IV)				
Sweden	Targeted antenatal		X			X	
	Routine (28–30 GW)		X (IM)			X (IM)	
	Postpartum		X (IM)			X (IM)	
The Netherlands[a]	Targeted antenatal	X					
	Routine (30GW)	X (IM)					
	Postpartum	X (IM)					
Belgium	Targeted antenatal			X			
	Routine (28 GW)			X (IM)			
	Postpartum			X (IM)			
Germany	Targeted antenatal		X			X	
	Routine (28–30 GW)		X (IM)			X (IM)	
	Postpartum		X (IM)			X (IM)	
Denmark	Targeted antenatal		X (> 20 GW)		X (< 20 GW)	X (> 20 GW)	
	Routine (29 GW)		X (IM)			X (IM)	
	Postpartum		X (IM)			X (IM)	
Ireland	Targeted antenatal		X (> 20 GW)		X (< 20 GW)	X (> 20 GW)	
	Routine (28–32 GW)		X (IM)				
	Postpartum		X (IM)			X (IM)	
Slovenia	Targeted antenatal		X (> 10 GW)		X (< 10 GW)	X (> 10 GW)	
	Routine (28–30 GW)		X (IM)			X (IM)	
	Postpartum		X (IM)			X (IM)	
Poland	Targeted antenatal		X			X	
	Routine (28–30 GW)		X (IM)			X (IM)	
	Postpartum						X (IM)
Italy[b]	Targeted antenatal		X			X	
	Routine (28 GW)		X (IM)			X (IM)	
	Postpartum		X (IM)			X (IM)	
Spain	Targeted antenatal		X				
	Routine (28 GW)		X (IM)				
	Postpartum		X (IM)				
Finland	Targeted antenatal		X (IM)			X (IM)	
	Routine (28–30 GW)		X (IM)			X (IM)	
	Postpartum		X (IM)			X (IM)	
France	Targeted antenatal	X					
	Routine (28 GW)		X (IM)				
	Postpartum	X (IV)					

GW, gestation week; IM, intramuscular; IU, international unit; IV, intravenous.

[a]RheDquin® used until 2016.

[b]Igamad®, Immunorho® and Partobulin® 300 µg (1500 IU) also used.

Table 2 Non-invasive foetal *RHD* genotyping and newborn RH1 phenotyping

Country	Earliest gestational age recommended for foetal *RHD* genotyping (GW)	Foetal DNA marker	Control of negative results	Programme government based	*RHD* genotype or RH1 phenotype for prophylaxis at birth	Cord blood RH1 phenotyping allowed	Recognition of the DVI antigen by the RH1 phenotyping reagent
Switzerland	18	No	Yes	No	RH1 phenotype	Yes	Yes
Sweden	10	No	No	Yes[a]	*RHD* genotype	Yes[b]	Yes (South) No (North)
The Netherlands	27	No	No	Yes	*RHD* genotype	Yes	Yes
Belgium	12	Yes	Yes	No	*RHD* genotype	Yes	Yes
Germany	12	Yes	No	Yes	RH1 phenotype	Yes	No
Denmark	22–25	No	Yes	Yes	*RHD* genotype	Yes	Yes
Ireland	11	No	Yes	No	RH1 phenotype	Yes	No
Slovenia	25	Yes	No	Yes	*RHD* genotype but control of positive results with RH1 phenotype	Yes	Yes
Poland	12	No	Yes	No	RH1 phenotype	Yes	Yes
Italy	18	No	Yes	No	RH1 phenotype	Yes	Yes
Spain	10	Yes	Yes	Yes	RH1 phenotype	Yes	Yes
Finland	24–26	No	No	Yes	*RHD* genotype	Yes	Yes
France	11	No	Yes	Yes	*RHD* genotype but control of negative results with RH1 phenotype	Yes	No[c]

GW, gestation week.

[a]Healthcare is regionalized; thus, decision how programme is designed decided by each region.

[b]Yes, but abolished in both regions.

[c]Reagents that recognize DVI antigen could be used but are not mandatory.

Table 3 Feto-maternal haemorrhage diagnosis and quantification

Country	Quantification of FMH in case of potential sensitizing event during pregnancy	Quantification of FMH at delivery	Test used to assess the FMH	Comments
Switzerland	No	No	/	Test performed during pregnancy (not at birth) only if there is a high risk of feto-maternal haemorrhage
Sweden	Yes	No	Kleihauer–Betke (microscopic analysis) or flow cytometry	Test only done if a clinically significant feto-maternal haemorrhage is suspected
The Netherlands	Yes	No	Kleihauer–Betke (microscopic analysis)	
Belgium	Yes	Yes	Kleihauer–Betke (microscopic analysis)	Test performed only after 20 GW
Germany	No	No	/	Test only done in rare uncommon case, where massive feto-maternal haemorrhage is suspected
Denmark	Yes / No	No	Flow cytometry	
Ireland	Yes	Yes	Kleihauer–Betke (microscopic analysis) or flow cytometry	
Slovenia	Yes	No	Kleihauer–Betke (microscopic analysis) or flow cytometry	
Poland	Yes	No	Flow cytometry	Test only done in rare uncommon case, where massive feto-maternal haemorrhage is suspected
Italy	No	No	/	
Spain	No	No	/	Test performed only in case of version or abdominal trauma
Finland	No	No	/	Test only done in rare uncommon case, where massive feto-maternal haemorrhage is suspected
France	Yes	Yes	Kleihauer–Betke (microscopic analysis) or flow cytometry	

FMH, feto-maternal haemorrhage.

Table 4 Antenatal and postnatal anti-RH1 prophylaxis

Country	Patients with a partial RH1 antigen (molecular characterization done) (sensitizing events, routine antenatal and at delivery anti-RH1 Ig administration)	Patients with a weak RH1 blood phenotype without molecular characterization (sensitizing events, routine antenatal and at delivery anti-RH1 Ig administration)	Routine antenatal Anti-RH1 Ig administration in case of undetermined foetal *RHD* genotype	Time limit for anti-RH1 Ig administration after delivery (h)	If the time limit is exceeded how long do you still administer anti-RH1 prophylaxis
Switzerland	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	72	2 weeks
Sweden	Same dose as RH1-negative patient	Same dose as RH1-negative patient[a]	Yes	72	No specification in the guidelines but generally 2 weeks
The Netherlands	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	48	No specification in the guidelines
Belgium	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	72	4 weeks
Germany	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	72	No specification in the guidelines
Denmark	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	72	No specification in the guidelines
Ireland	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	72	10 days
Slovenia	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	72	2 weeks
Poland	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	72	2 weeks
Italy	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	72	1 week
Spain	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	72	No specification in the guidelines
Finland	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	72	2 weeks
France	Same dose as RH1-negative patient	Same dose as RH1-negative patient	Yes	72	4 weeks

[a]Sweden: it depends on the weakness of the phenotype (if at least 3 + or 4 + reactions by antiglobulin test with the two selected anti-RH1 reagents, patients are considered as RH1 positive).

Table 5 Measure of the efficacy of immunoprophylaxis

Country	After anti-RH1 Ig administration : looking for the reversion of the Kleihauer test if positive	Antibody screen within a few days after anti-RH1 Ig administration	Antibody screen sometime after delivery	How much time after
Switzerland	No	Yes	No	
Sweden	No	Yes (South) / No (North)	No (except if FMH)	
The Netherlands	No	No	No	
Belgium	Yes	Yes	Yes	1–3 months
Germany	No	No	No (except massive FMH)	
Denmark	No	No	No	
Ireland	Yes	No	Yes	6 weeks
Slovenia	Yes	Yes	Yes	
Poland	No	No	Yes	6 weeks
Italy	Yes	No	Yes	6 months
Spain	No	No	No	
Finland	No	No	No	
France	yes	No	Yes	6 months

FMH, feto-maternal haemorrhage.

6 countries ask systematically for a screening control a certain time period after delivery. Two more countries recommend an antibody screening control but only in cases of massive FMH (Table 5).

Because of anti-RH1 Ig administration, it is sometimes difficult to distinguish between passive and immune anti-RH1 at the indirect antiglobulin tests (IAT) performed during pregnancies. We asked about the countries strategy to interpret the IAT. Almost all countries (12) are always performing a screening test just before anti-RH1 Ig administration. For the IAT done after anti-RH1 Ig administration, Ireland, Germany, Belgium and France have developed specific quantitative tests to distinguish between passive and immune anti-RH1, based on the pharmacokinetic of anti-RH1 Ig treatment [6]. It can be titration with a column agglutination test, quantification with an anti-RH1 standard and a continuous flow haemagglutination assay on an autoanalyzer, or microtitration with an anti-RH1 standard on a column agglutination test. Six countries prefer the strategy of performing repeated titration test to have a kinetic monitoring of anti-RH1 concentrations (Table 6).

To record the number of errors or omissions of anti-RH1 Ig treatment, Ireland, Slovenia, Spain and the Netherlands have set up a haemovigilance scheme, even if in most of these countries, the reports seem not to be exhaustive (Table 7).

A national register to monitor the incidence of anti-RH1 alloimmunized pregnant women has been established in Denmark, Slovenia and Finland. In other countries, periodic surveys are also highlighting the efficacy of routine antenatal immunoprophylaxis and the very low level of the actual anti-RH1 immunization rates (Table 7).

Discussion

Most of the survey participants have national guidelines. All of them recommended targeted and routine antenatal immunoprophylaxis, as well as postpartum anti-RH1 Ig administration, in accordance with the World Health Organization recommendations.

Anti-RH1 Ig Products used in Europe may differ but three different drugs are preponderant: Rhophylac® (CSL Behring), Rhesonativ® (Octapharma) and RhoGAM® (Kedrion S.p.A). They are all polyclonal human Ig and equivalent in terms of dosing and bioavailability. They are manufactured in countries allowing hyperimmunizations of volunteered donors which raises ethical issues. It could also induce difficulties for some countries to obtain sufficient supplies of anti-RH1 Ig. The perspective of monoclonal anti-RH1 Ig is still pending: it would however address both of these issues.

The dose used in European countries is similar, between 1000 and 1500 IU depending on the drug and the type of prophylaxis. Low-dose 625 IU Rhesonativ® may be used for early gestational ages as the embryonic or foetal blood volume is very low.

IM route is preponderant probably because Rhesonativ® and RhoGAM® are licensed for IM injection only. The bioavailability of the anti-RH1 Ig is however not the same, depending on the route used: IV injections are associated with higher anti-RH1 concentrations in the first days after anti-RH1 Ig administration [6]. Thus, the IV route may induce a faster clearance of foetal RH1-positive red blood cells in the maternal blood.

All the countries that have responded use a one-dose regimen for routine anti-RH1 Ig administration (1000 or

Table 6 Detection of anti-RH1 immunization despite immunoprophylaxis

Country	Performance of a red cell antibody screening before anti-RH1 Ig administration	Specific test for low-level anti-RH1 quantification	Type of specific test	Other strategy to distinguish between passive and immune anti-RH1 antibodies
Switzerland	Yes	No	/	
Sweden	Yes	No	/	Repeated titration to have a kinetic monitoring of the anti-RH1 concentration
The Netherlands	Yes	No	/	Repeated titration to have a kinetic monitoring of the anti-RH1 concentration
Belgium	Yes	Yes	Anti-RH1 microtitration (with anti-RH1 standard on column agglutination technology)	
Germany	Yes	Yes	Anti-RH1 titration (column agglutination technology)	Repeated titration to have a kinetic monitoring of the anti-RH1 concentration
Denmark	Yes	No	/	Repeated titration to have a kinetic monitoring of the anti-RH1 concentration
Ireland	Yes	Yes	Anti-RH1 quantification (Continuous Flow Analysis on Autoanalyzer)	
Slovenia	Yes	No	/	
Poland	Yes	No	/	Repeated titration to have a kinetic monitoring of the anti-RH1 concentration
Italy	Yes	No	/	
Spain	Yes	No	/	Repeated titration to have a kinetic monitoring of the anti-RH1 concentration
Finland	No	No	/	Repeated titration to have a kinetic monitoring of the anti-RH1 concentration
France	Yes	Yes	Anti-RH1 microtitration (with anti-RH1 standard on column agglutination technology)	Repeated titration to have a kinetic monitoring of the anti-RH1 concentration

Table 7 Impact of anti-RH1 prophylaxis on anti-RH1 sensitizations

Country	Haemovigilance scheme to collect errors in anti-RH1 Ig administration	Exhaustivity of the haemovigilance scheme	National register to monitor the incidence of anti-RH1 alloimmunized pregnant women	Regular evaluation of the impact of the anti-RH1 prophylaxis program
Switzerland	No	/	No	No
Sweden	Yes (South)/ No (North)	No	No (South)/ Yes (North)	Yes
The Netherlands	Yes	No	No	No
Belgium	No	/	No	No
Germany	No	/	No	No
Denmark	No	/	Yes	Yes
Ireland	Yes	No	No	No
Slovenia	Yes	Yes	Yes	Yes
Poland	No	/	No	No
Italy	No	/	No	No
Spain	Yes	No	No	No
Finland	No	/	Yes	Yes
France	No	/	No	No

1500 IU around 28 GW). The two-dose regimen (500 IU at 28 then at 34 GW) was used until 2015 in the UK and is still recommended in some non-European countries like Australia or New Zealand [7]. Both approaches seem to be equally effective [3,8] but compliance may be more difficult to obtain with the two-dose regimen, even if improvements have been done in those countries with increasing experience and education [9,10].

Foetal *RHD* genotyping has been implemented in all participating countries. The systematization of the test is especially seen in countries where the costs of the programme are fully covered by the government or by local institutions. In countries where this test has been institutionalized, the compliance with the prevention programme appears to be high [11].

There was no question concerning the number and type of amplified exons for foetal *RHD* genotyping, but most of the European countries are using at least 2 exons to interpret the genotype results. The accuracy of the test is high regardless the methodology used [12,13]. Detecting at least two exons is actually recommended to increase the chance to detect *RHD* variants. Cost-effectiveness studies give different results: these discrepancies could be partly explained depending on whether or not the genotype result is used for postnatal immunoprophylaxis. Globally, it appears that performing foetal *RHD* genotyping to guide anti-RH1 Ig administration is less or equally expensive than administering systematically anti-RH1 Ig to all RH1-negative pregnant women. This strategy also avoids anti-RH1 Ig overuse and 'out of stock' problems. It also seems that the performance of managing appropriately anti-RH1 prophylaxis was better if a foetal *RHD* genotyping is performed [14]. Lastly, it is a better ethical approach, as 40% of pregnant RHD-negative women would not unnecessarily be given a blood product [15].

This European approach proves to be efficient [16] but differs from that of the USA. Indeed, overseas, foetal *RHD* genotyping is not frequently performed. Because of the high percentage of *RHD* variants linked to a high ethnic diversity in the USA's population, the gold standard to be sure to avoid false-negative results and maternal anti-D sensitization remains RH1 phenotyping [17]. However, it is likely that the accuracy of foetal RHD genotyping may be underrated because it is often assumed that the accuracy of RH1 phenotyping is 100%. In a recent study, it was reported that additional serological and molecular testing of cord blood samples typed as RH1 negative when the results of foetal *RHD* testing were positive showed that in 0·09% of cases, cord blood serology was in fact false negative [18].

In European countries, even if false-negative genotyping results are at very low rates [19,20], to further limit the risk, different strategies emerge. The attractive idea of using a foetal DNA marker in the foetal genotyping test is not so easy to apply [12]. There is no foetal marker that can be easily implemented in a screening setting: detection of epigenetic markers as RASSF1a is associated with low sensitivity and specificity, and amplification of paternally inherited markers is not feasible in high-throughput routine use. Most of laboratories are considering such a control as not necessary for non-invasive foetal *RHD* genotyping due to high sensitivities reported with the methods [13,21]. Nevertheless, European countries proposed different alternatives to ensure a near 100% sensitivity, that is to perform the test at the beginning of the third trimester [18,22], to control all negative results antenatally on another maternal blood samples or to do a phenotype at birth on the cord or on the newborn's blood. It is also a cost-effectiveness issue, and it explains why strategies may differ among countries [14,23].

For most of the countries performing RH1 phenotyping of the newborn, the reagent used must recognize the DVI antigen. DVI is indeed the most frequent RH1 partial antigen among European populations. This variant is considered immunogenic as DVI donors have to be treated as D positive in most of European guidelines [5].

An FMH of more than 15 ml of foetal red cells, when more than 1000 to 1500 IU of anti-RH1 Ig are needed, is a very rare event (approximately 0·3 % of pregnancies) [24]. And not all women with such FMH will develop anti-RH1 alloimmunization. This could explain why systematic detection of FMH before targeted immunoprophylaxis or after delivery is not performed in all countries. In some countries, FMH screening tests are realized only following events potentially associated with large bleedings caused by placental trauma and disruption of the feto-maternal interface (e.g. abdominal trauma during the third trimester, external cephalic version, intrauterine deaths, foetal anaemia, stillbirth or instrumental or caesarean section at delivery). However, up to 50% of large FMHs at delivery occur in women without identifying risk factors [25].

Concerning anti-RH1 Ig administration for pregnant women with a partial or non-characterized *RHD* variant allele, all countries adopt the same strategy, which is to consider them as RH1-negative patient, both for targeted and routine antenatal prophylaxis. One can think that the efficient dose of anti-RH1 Ig may be different for those women and even differ between *RHD* variant alleles. The strategy to adopt for these women concerning routine anti-RH1 Ig may also have to be reconsidered, because it is likely that the dose of anti-RH1 Ig administered will not be sufficient to cover small 'silent' FMH during the

whole third trimester of pregnancy. More studies may be required in this field.

If all countries have a time limit of 48 or 72 h to administer anti-RH1 Ig after birth, the management of the situation, when the prophylaxis has been omitted, varies among countries with a possibility to administer anti-RH1 Ig until 1 to 4 weeks after delivery. There is also here a lack of consensus in the guidelines certainly because of a reduced number of studies in this area.

Although Rh disease prevention policies were very efficient for more than 50 years in Europe (drastic reduction in the immunization rate), HDFN cases due to maternal anti-RH1 immunization have not completely disappeared. It seems that errors or omissions of anti-RH1 Ig administration [26], massive FMH or FMH not covered at the correct time or with sufficient anti-RH1 Ig [27,28], or a previous pregnancy abroad without prophylaxis (unpublished data of the French National Reference Center in Perinatal Hemobiology) may be the major causes of the remaining anti-RH1 sensitization cases.

Recently, efforts have been done to try to limit the use on anti-RH1 Ig only to pregnant women for whom it is necessary, thanks to foetal *RHD* genotyping. Even if the approaches may differ between countries, all of them have undertaken some measures to try to limit the incidence of remaining immunization cases.

European countries are also trying to monitor RH disease prevention efficacy, but in different ways. Immediate monitoring during pregnancies or just after delivery could be performed through the follow-up of the reversion of the Kleihauer test in case of FMH, checking for the negativity of the IAT before anti-RH1 Ig administration, or for its positivity after anti-RH1 Ig injection, having a quantitative approach of circulating anti-RH1 when present at the IAT [29,30] and performing a screening test control a few weeks or months after delivery.

For long-term monitoring, whereas some countries have set up a solid haemovigilance scheme or established a national register to report the remaining immunization cases, others are following up the efficacy of their prevention policy through regular periodical epidemiological studies.

This survey highlights the good practices undertaken in different European countries. It is interesting to see that several different but efficient strategies are emerging. Sharing them may allow continuous improvement in reducing anti-RH1 alloimmunization cases.

Conflict of interest

The authors declare no conflict of interests.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Questionnaire.
Appendix S2. References of the National guidelines.