



# The Effectiveness and Compliance of Routine Antenatal RhIG Prophylaxis using Different Strategies: A Systematic Review and Meta-analysis

Kar Men Tee<sup>1</sup>, Denise E Jackson<sup>1,2\*</sup>

<sup>1</sup>Department of Health and Biomedical Sciences, RMIT University, Bundoora, Victoria, Australia; <sup>2</sup>Department of Laboratory Medicine, RMIT University, Bundoora, Victoria, Australia

## ABSTRACT

Routine antenatal anti-D prophylaxis has successfully reduced the incidence of RhD alloimmunisation and Haemolytic Disease of the Foetus and Newborn (HDFN). However, there are different strategies and guidelines available across different countries. This systematic review and meta-analysis aimed to investigate the effectiveness and compliance of routine antenatal prophylaxis of different strategies by measuring detectable anti-D levels at delivery, RhD alloimmunisation rate and compliance. PubMed, SCOPUS, EMBASE and Cochrane Library were searched up until 30 July 2023. Binary random effects meta-analysis using maximum likelihood method was performed in Open Meta Analyst software. 17 studies were eligible for meta-analysis. More women had detectable anti-D at delivery after two-dose regimen (24.5%; estimate 0.532, 95% CI [0.181, 0.866],  $P < 0.001$ ) than one-dose regimen (18.35%; estimate 0.252, 95% CI [0.107, 0.433],  $P < 0.001$ ). The rate of RhD alloimmunisation was higher for two-dose regimen (0.33%; estimate 0.003, 95% CI [0.002, 0.005],  $P < 0.001$ ) when compared to one-dose regimen (0.13%; estimate 0.002, 95% CI [0.000, 0.005],  $P < 0.001$ ). Targeted antenatal Rh Immune Globulin (RhIG) had the highest compliance (90.01%; estimate 0.903, 95% CI [0.880, 0.924],  $P < 0.001$ ). One-dose had higher compliance when compared to two-dose regimens (88.73% vs. 61.49%;  $P = 0.004$ ). Both universal one-dose and two-dose regimens were equally effective for the prevention of RhD alloimmunisation. However, one-dose regimen would perform better than two-dose regimen due to higher compliance. The targeted regimen which uses a single dose of RhIG is considered the best strategy for routine antenatal prophylaxis with the highest compliance.

**Keywords:** Routine antenatal prophylaxis; Rhig; One-dose; Two-dose; Targeted; Detectable anti-D; RhD alloimmunisation; Compliance

## INTRODUCTION

Since the introduction of Rhesus D (RhD) Immunoglobulin (Ig) in the late 1960s, the prevalence of anti-D mediated Haemolytic Disease of the Foetus and Newborn (HDFN) and RhD alloimmunisation has significantly declined [1]. HDFN is a condition known as the destruction of foetal/neonatal erythroid cells caused by RhD alloimmunisation in RhD-negative women, which may result in anaemia, jaundice, kernicterus, hydrops foetalis, stillbirth and neonatal death in severe cases [2]. Rh Immunoprophylaxis (RhIG) is produced by purifying human polyclonal anti-D Immunoglobulin G (IgG) from pooled plasma of hyperimmunised male donors. RhIG was first introduced for postpartum administration, where a standard dose is given within 72 hours to all non-sensitised RhD-negative women after delivery of RhD-positive infant.

Postnatal administration of RhIG successfully reduced the risk of Rh alloimmunisation from 13% to 1-2% [3]. However, studies

reported failure of postnatal RhIG due to sensitising events during pregnancy and highlighted the potential of antenatal RhIG [4,5]. Some studies demonstrated the likelihood of sensitising events during the third trimester of pregnancy without visible clinical symptoms [6-9]. Antenatal prophylaxis was proposed with the combined use of postnatal prophylaxis and further reduced the overall sensitisation rate to 0.07%-0.3% [10-15]. Many countries, mainly developed countries, have implemented different guidelines and strategies for routine antenatal prophylaxis [9,16].

### Routine antenatal anti-D prophylaxis

The purpose of antenatal prophylaxis is to administer RhIG to all non-immunised RhD-negative pregnant women during pregnancy who have the risk of RhD alloimmunisation regardless of the foetal RhD status. The burden of HDFN is most prevalent in Caucasians with an estimated 15% of the population being RhD-negative [17].

**Correspondence to:** Denise E Jackson, Department of Laboratory Medicine, RMIT University, Bundoora, Victoria, Australia, E-mail: denise.jackson@rmit.edu.au

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Of all pregnancies, there are approximately 10% RhD-negative women delivered an RhD-positive foetus with the risk of sensitisation by Fetomaternal Haemorrhage (FMH) [7]. Prevention of RhD alloimmunisation by RhIG usually focuses on the first pregnancy to avoid complications in subsequent pregnancies with an increased risk of HDFN. In the first pregnancy, approximately 60% RhD-negative women will deliver an RhD-positive infant [7]. Development of anti-D IgG antibodies requires a transplacental haemorrhage sufficient to trigger an immune response, where the majority occurs after 28th week of gestations [9]. Thus, antenatal RhIG is administered routinely in the third trimester of pregnancy to suppress potential RhD alloimmunisation by passively removing any foetal D antigen in the mother [6]. The possible routes of administration are either intramuscularly or intravenously, where intramuscular injections are more commonly used.

### Universal one-dose vs. two-dose regimens

There is no one standardised routine antenatal prophylaxis programme globally. The British Committee for Standards in Haematology (BCSH) guidelines and the Society of Obstetricians and Gynaecologists of Canada (SOGC) recommend either one 1500 International Units (IU) at 28 weeks of gestation or 500 IU doses at 28 and 34 weeks [18,19]. The American Congress of Obstetricians and Gynecologists (ACOG) recommends administering a single 1500 IU dose at 28 weeks of gestation [20]. The Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) recommend 625 IU doses at 28 and 34 weeks [21]. The National Prevention Program of the Netherlands recommends 1000 IU at 30 weeks' gestation, Germany routinely administers a single 300 µg dose at 28-30 weeks' gestation, and different strategies for other European countries [8,14,22]. Therefore, the two main approaches for antenatal administration of RhIG are either the one-dose regimen of 1000 IU-1500 IU dose between 28-30 weeks' gestation or the two-dose regimen of 500-625 IU doses at 28 and 34 weeks' gestation.

### Targeted regimens

Recently, many have suggested prenatal screening of foetal *RHD* genotype using a Non-Invasive Prenatal Test (NIPT) to guide targeted administration of antenatal RhIG. In the late 1990s, Lo and his colleagues first discovered low concentrations of cell-free foetal Deoxyribonucleic acid (cfDNA) present in maternal plasma without the use of invasive procedures [23,24]. Detection of the foetal *RHD* gene in maternal plasma using a whole Ethylenediaminetetraacetic Acid (EDTA) blood sample provides high sensitivity and accuracy. This new regimen can avoid unnecessary exposure to RhIG derived from human plasma, reduce wasteful use of RhIG and increase the utilisation of RhIG to only RhD-negative women pregnant with an RhD-positive foetus [25]. After the success of further clinical trials, Denmark was the first country to implement a national prenatal screening for foetal *RHD* before the administration of antenatal RhIG in January 2010 [26]. Followed by the Netherlands in July 2011, Finland in October 2013, Norway in September 2016 as well as regional Sweden [27-31]. The general practice is a NIPT of all non-immunised RhD-negative pregnant women at 24 weeks' gestation followed by a single 1250-1500 IU dose at 28-30 weeks to RhD-negative pregnant women carrying a RhD-positive foetus [25,29]. Australia is still evaluating the feasibility of introducing routine NIPT as part of antenatal care [32].

### Scope of study

Several strategies are available: universal one-dose, universal two-dose, and the latest targeted regimens, where different countries and locations

adopted different regimens. Some suggested the one-dose regimen is superior to the two-dose regimen while others suggested otherwise. Despite the efforts of routine antenatal and postnatal prophylaxis, there are residual cases of RhD alloimmunisation. Compliance with routine antenatal prophylaxis regimen may contribute to failure of the regimens. Missing or incorrect doses of routine antenatal prophylaxis or given at the wrong timing may put non-immunised RhD-negative pregnant at risk of RhD alloimmunisation. Therefore, this study aims to investigate the following primary question: Is the one-dose antenatal anti-D prophylaxis regimen more effective than the two-dose regimen in protecting non-immunised RhD-negative pregnant women against RhD immunisation? With a secondary question: Does the one-dose regimen have higher compliance than two-dose routine antenatal anti-D prophylaxis? An addition question: How does targeted regimen perform when compared to one-dose and two-dose regimen? This study evaluates the effectiveness and compliance of routine antenatal prophylaxis of different strategies for the prevention of RhD alloimmunisation and HDFN with regard to the level of detectable anti-D at delivery, the rate of RhD alloimmunisation/sensitisation after delivery, and the rate of compliance.

## MATERIALS AND METHODS

This meta-analysis was prepared according to the protocols outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [33].

### Search strategy and study selection

To identify eligible literature for this meta-analysis, the following databases were systematically searched for: PubMed, SCOPUS, EMBASE and Cochrane Library. The latest search was conducted on 30<sup>th</sup> July 2023. The following keywords and phrases in various combinations were used in accordance with the search strategy: anti-D prophylaxis, RhIG prophylaxis, antenatal RhIG, antenatal prophylaxis, anti-D immunoglobulin, RhD immune globulin, Rhesus prophylaxis, single-dose, one-dose, and two-dose. Additional literature was included by hand searching reference lists of potential articles. Articles were retrieved based on the search strategy and duplicates were removed. The titles and abstracts were screened for relevancy based on the predefined exclusion and inclusion criteria to identify potentially eligible studies for meta-analysis.

### Exclusion and inclusion criteria

Eligible studies must include non-immunised RhD-negative pregnant women given one dose, two doses, or targeted routine antenatal RhIG intramuscularly during pregnancy. Studies should investigate a single dose of 1500 IU administered at 28-30 weeks' gestation for one-dose regimen, two doses of 500-625 IU administered at 28 and 34 weeks' gestations for two-dose regimen, and a single 1250 IU-1500 IU administered at 28-30 weeks' gestation guided by a NIPT for targeted regimen. Review articles, guidelines, meta-analyses, editorials, letters, and case reports were excluded. Non-English articles without translation available were excluded. Any studies that only evaluated the effect of postnatal prophylaxis, RhIG given antenatally due to complicated pregnancies, or intravenous administration were excluded. Abstract-only publications and full text not available were excluded.

### Quality assessment of eligible studies

All eligible studies were assessed for reporting quality using the recommendations outlined in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [34].

## Data extraction

Information on study design, location, study period, sample size, type of regimen and measurements were extracted to summarise the characteristics of each eligible study. Relevant data were extracted from each eligible study and grouped based on the measurements for further investigation and comparison.

## Statistical analysis

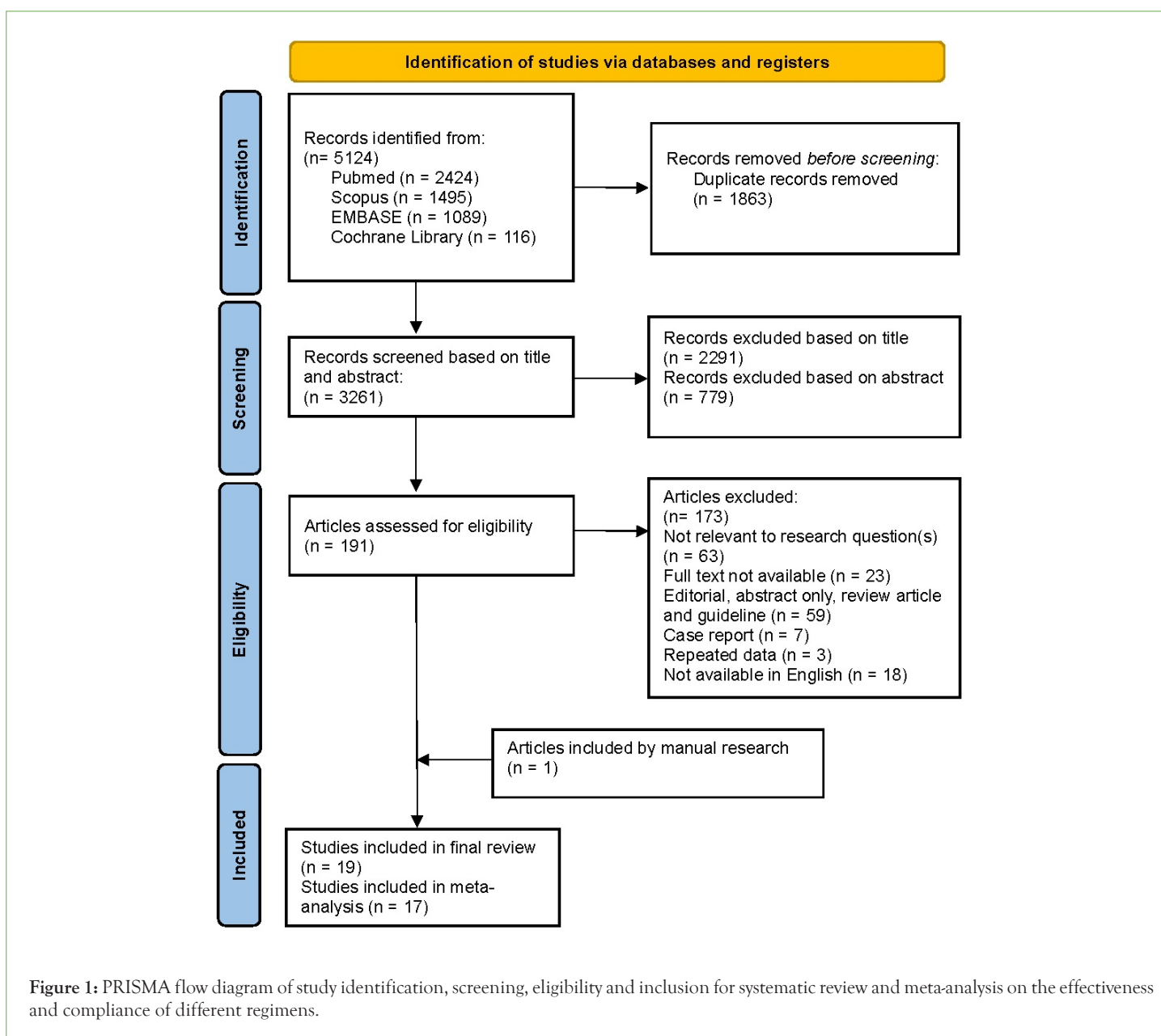
Extracted data was analysed in Open Meta Analyst software (version 12.11.14) from the Brown University website, and results were presented in forest plots. One-arm using arcsine transformed proportion metric were employed to explore the effects of detectable anti-D, RhD alloimmunisation and compliance of one-dose, two-dose and targeted regimens. Two-arm using difference of arcsine transformed proportions metric was employed to explore the relationship of a single measurement between regimens, if applicable. For both metrics, a binary random effects meta-analysis was conducted using maximum likelihood method. Multiple forest plots were generated, displaying the estimated 95% confidence intervals, P-values, and heterogeneity for

each study and overall pooled effect of meta-analysis. The magnitude of heterogeneity was measured as I<sup>2</sup> statistic, where below 40% may suggest not important heterogeneity, 30%-60% suggest moderate heterogeneity and above 50% suggest substantial heterogeneity [35]. Statistical significance was expressed as P-value, where  $P \leq 0.05$  defined as 'statistically significant'.

## RESULTS

### Study selection

Overall, 5124 citations were retrieved from PubMed, SCOPUS, EMBASE and Cochrane Library. After removing 1863 duplicates, 2291 articles were excluded by title screening and 779 based on abstract, yielding a total of 191 articles to assess for eligibility. Of the 191 articles, 173 were excluded based on the predefined exclusion criteria and 18 studies were included after final review. One additional eligible article was manually searched from reference lists. A total of 19 studies were included in this review but only 17 were included for meta-analysis (Figure 1).



### Study characteristics

As shown in Table 1, the eligible studies included nine retrospective studies four prospective studies five clinical trials and one did not

specify its study design. These studies were conducted across eight countries in 1975-2019. One study was missing information on study period [22].

**Table 1:** Characteristics of eligible studies for the analysis of effectiveness and compliance of different regimens.

Study	Country	Study Design	Study Period	Study Size	Regimen	Measure(s)
Bowman and Pollock; 1978 [36]	Canada	Clinical trial	1975	1086	One-dose	RhD alloimmunisation
Chaffe et al.; 2007 [37]	UK	Retrospective audit	2004	207	Two-dose	Compliance
Clausen et al.; 2014 [38]	Denmark	Retrospective	2010-2012	690	Targeted	Compliance
Damkjaer et al.; 2012 [39]	Denmark	Retrospective	2010	239	Targeted	Compliance
Davies et al.; 2011 [8]	UK	Retrospective	2009	157	One-dose	Detectable anti-D
				407	Two-dose	Detectable anti-D
Glazebrook et al.; 2020 [40]	Australia	Retrospective	2017-2018	939	Two-dose	Compliance
MacKenzie et al.; 1999 [41]	UK	Prospective	1990-1996	3332	One-dose	RhD alloimmunisation
MacKenzie et al.; 2004 [22]	UK, USA	Clinical trial	-	216	Two-dose	RhD alloimmunisation
MacKenzie et al.; 2006 [42]	UK	Prospective	1992-2003	546	Two-dose	Compliance
MacKenzie et al.; 2011 [43]	UK	Prospective	2009	144	One-dose	Compliance
				500	Two-dose	Compliance
Mayne et al.; 1997 [13]	UK	Retrospective	1993-1995	1425	Two-dose	RhD alloimmunisation
Rowley et al.; 2014 [44]	UK	Retrospective audit	2012	4887	One-dose	Compliance
				389	Two-dose	Compliance
Rudensky et al.; 2003 [45]	Israel	-	2000	150	One-dose	Detectable anti-D
Sorensen et al.; 2022 [30]	Norway	Retrospective	2017-2019	984	One-dose	Detectable anti-D
Tiblad et al.; 2013 [25]	Sweden	Prospective	2009-2012	9380	Targeted	RhD alloimmunisation, Compliance
Tovey et al.; 1983 [11]	UK	Clinical trial	1980-1981	2069	Two-dose	Detectable anti-D, RhD alloimmunisation
Trolle 1989 [46]	Denmark	Clinical trial	1980-1985	609	One-dose	Detectable anti-D, RhD alloimmunisation
White et al.; 2019 [47]	Australia	Randomised clinical trial	2013-2015	138	One-dose	Detectable anti-D, Compliance
				139	Two-dose	Detectable anti-D, Compliance
Wikman et al.; 2021 [48]	Sweden	Retrospective	2010-2012	4280	Targeted	Detectable anti-D

For the detection of anti-D at delivery, two studies used the DiaMed gel method, one study used papain-treated antibody screen with passive anti-D quantified as <0.1 IU/mL, one study used Ortho BioVue column agglutination technique, and one study did not specify the technique for antibody screen [8, 11,45-47]. To assess the rate of RhD alloimmunisation, women who delivered a RhD-positive infants were followed up in 6-12 months in three studies [22,36,46]. Besides, two studies measured sensitised second pregnancies, and one study followed up to 12 months after delivery and subsequent sensitised second pregnancies [11,13,41].

To assess compliance, the definition of 'appropriate timing' for injection slightly varied between studies. For one-dose regimen, two studies assessed injection given at 28 weeks' gestation with 2 weeks intervals, while one study assessed injection given within a week of 28 weeks' gestation [43,44,47]. For two-dose regimen, two studies assessed injections given within a week of 28 and 34 weeks' gestation, two studies assessed first injection at 28 ± 1 weeks' gestation and second injection given after 6 ± 1 weeks, and other two studies given with a 2-week interval for both injections (28-30 and 34-36 weeks' gestation) [37,40,42-44,47]. However, targeted regimen had a broader definition for compliance, which was assessed by the total number of RhD-negative pregnant women carrying a RhD-positive foetus received recommended antenatal prophylaxis [25,38,39]. Each included study recorded at least one measurement for effectiveness and/or compliance (Table 2).

**Table 2:** Data of included studies in meta-analysis portraying in proportions.

Study	Regimen	Detectable anti-D at delivery, n/N	RhD alloimmunisation, n/N	Compliance, n/N
Bowman and Pollock; 1978 [36]	One-dose	-	2/1086	-
Chaffe et al.; 2007 [37]	Two-dose	-	-	179/207
Clausen et al.; 2014 [38]	Targeted	-	-	330/354
Damkjaer et al.; 2012 [39]	Targeted	-	-	126/147
Davies et al.; 2011 [8]	One-dose	34/157	-	-
	Two-dose	247/407	-	-
Glazebrook et al.; 2020 [40]	Two-dose	-	-	681/920
MacKenzie et al.; 1999 [41]	Two-dose	-	12/3320	-
MacKenzie et al.; 2004 [22]	One-dose	-	0/194	-
MacKenzie et al.; 2006 [42]	Two-dose	-	-	232/401

MacKenzie et al.; 2011 [43]	One-dose	-	-	108/139
	Two-dose	-	-	304/451
Mayne et al.; 1997 [13]	Two-dose	-	4/1425	-
Rowley et al.; 2014 [44]	One-dose	-	-	4388/4887
	Two-dose	-	-	228/389
Rudensky et al.; 2003 [45]	One-dose	32/150	-	-
Tiblad et al.; 2013 [25]	Targeted	-	14/9380	4590/5104
Tovey et al.; 1983 [11]	Two-dose	282/2069	4/1238	-
Trolle 1989 [46]	One-dose	55/609	0/291	-
White et al.; 2019 [47]	One-dose	70/125	-	86/138
	Two-dose	111/129	-	70/139

**Note:** N = total number of cases tested for the respective measurements; n = number of cases identified for the respective measurements.

### Study quality assessment

The included studies were assessed for their methodological quality according to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines, with the most relevant criteria selected and summarised in Table 3. Of 19 included studies, only nine studies had fulfilled all criteria and were considered as high quality [22,25,30,39,40,43,44,47,48]. The remaining ten studies were considered of lower quality as most studies failed to describe statistical methods and address potential limitations. However, two studies were further identified as poor quality due to only stating rationale without explaining the background of the study and the lack of an abstract in one of them [13,45]. Studies that did not describe statistical methods and discuss potential limitations were mostly conducted in the late 1900s and early 2000s, except the study by MacKenzie and colleagues which was conducted in 2004 [8,11,13,22,36-38,41,42,45,46]. A possible reason could be that guidelines and practices for standardised reporting may not be well established and with poor adherence. However, the studies conducted in the early period provided the fundamental evidence used to form the early guidelines for the national routine antenatal prophylaxis programme. Thus, considering the impact of these early studies, some studies of lower quality were still included in this meta-analysis.

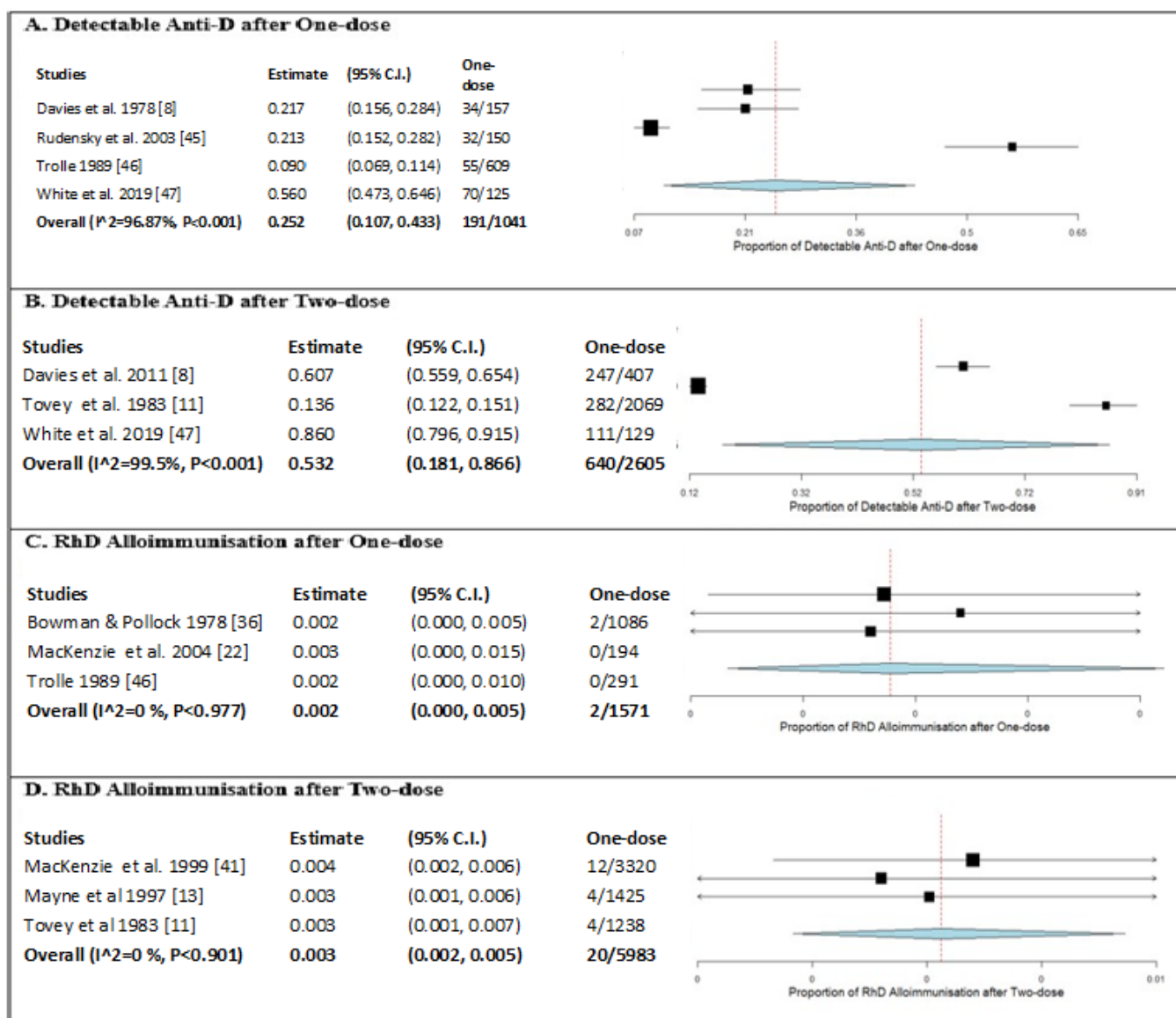
### Meta-analysis of detectable anti-D at delivery

Meta-analysis on detectable anti-D at delivery was performed for one-dose and two-dose regimens, with results presented in forest plots (Figure 2). The one-arm proportion analyses were statistically significant for one-dose (estimate 0.252, 95% CI [0.107, 0.433],  $P < 0.001$ ), and two-dose regimens (estimate 0.532, 95% CI [0.181, 0.866],  $P < 0.001$ ). The overall results showed that 18.35% of women had detectable anti-D after one-dose regimen, while 24.57% of women had detectable anti-D after two-dose regimen. The results were highly heterogeneous for the one-dose regimen ( $I^2 = 96.87\%$ ,  $P < 0.001$ ) and for the two-dose regimen ( $I^2 = 99.50\%$ ,  $P < 0.001$ ), indicating variability in data for studies included in the meta-analyses. Overall, the results suggested that two-dose regimen had higher detectable anti-D at delivery compared to one-dose regimen.

**Table 3:** Evaluation of methodological quality of eligible studies according to the STROBE checklist.

	Title and abstract	Introduction	Methods	Results	Discussion		
	Clear title and abstract with information on study design	Explain scientific background and rationale	Provide detailed study method	Provide eligibility criteria and selection of participants	Describe statistical methods	Report numbers of individuals at each stage of study	Summarise key results and discuss potential limitations
Bowman and Pollock; 1978 [36]	Y	Y	Y	Y	N	Y	N <sup>a</sup>
Chaffe et al.; 2007 [37]	Y	Y	Y	Y	N <sup>b</sup>	Y	N <sup>a</sup>
Clausen et al.; 2014 [38]	Y	Y	Y	Y	Y	Y	N <sup>a</sup>
Dankjaer et al.; 2012 [39]	Y	Y	Y	Y	Y	Y	Y
Davies et al.; 2011 [8]	Y	Y	Y	Y	Y	Y	N <sup>a</sup>
Glazebrook et al.; 2020 [40]	Y	Y	Y	Y	Y	Y	Y
MacKenzie et al.; 1999 [41]	Y	Y	Y	Y	Y	Y	N <sup>a</sup>
MacKenzie et al.; 2004 [22]	Y	Y	Y	Y	Y	Y	Y
MacKenzie et al.; 2006 [42]	Y	Y	Y	Y	N	Y	N <sup>a</sup>
MacKenzie et al.; 2011 [43]	Y	Y	Y	Y	Y	Y	Y
Mayne et al.; 1997 [13]	N <sup>c</sup>	N <sup>d</sup>	Y	Y	N	Y	N <sup>a</sup>
Rowley et al.; 2014 [44]	Y	Y	Y	Y	Y	Y	Y
Rudensky et al.; 2003 [45]	Y	N <sup>d</sup>	Y	Y	N	Y	N <sup>a</sup>
Sorensen et al.; 2022 [30]	Y	Y	Y	Y	Y	Y	Y
Tiblad et al.; 2013 [25]	Y	Y	Y	Y	Y	Y	Y
Tovey et al.; 1983 [11]	Y	Y	Y	Y	N	Y	N <sup>a</sup>
Trolle 1989 [46]	Y	Y	Y	Y	N	Y	N <sup>a</sup>
White et al.; 2019 [47]	Y	Y	Y	Y	Y	Y	Y
Wikman et al.; 2021 [48]	Y	Y	Y	Y	Y	Y	Y

**Note:** Y = criteria fulfilled; N = criteria unfulfilled. <sup>a</sup> No limitation discussed; <sup>b</sup> Audit standards were defined; <sup>c</sup> No abstract included; <sup>d</sup> Only stated the rationale.



**Figure 2:** Forest plots of meta-analysis on the effectiveness of routine antenatal prophylaxis: The detectable anti-D antibody at delivery. (A) after one-dose regimen; (B) after two-dose regimen, and the incidence of RhD alloimmunisation; (C) after one-dose regimen; (D) after two-regimen. **Note:** Results were generated using arcsine transformed proportion analysis with maximum likelihood estimation of binary random effects model, and pooled effect size was estimated with 95% confidence. Statistical significance was determined using P-value; heterogeneity was expressed as I<sup>2</sup> with associated P-value. The weighting was determined from the sample size of each study.

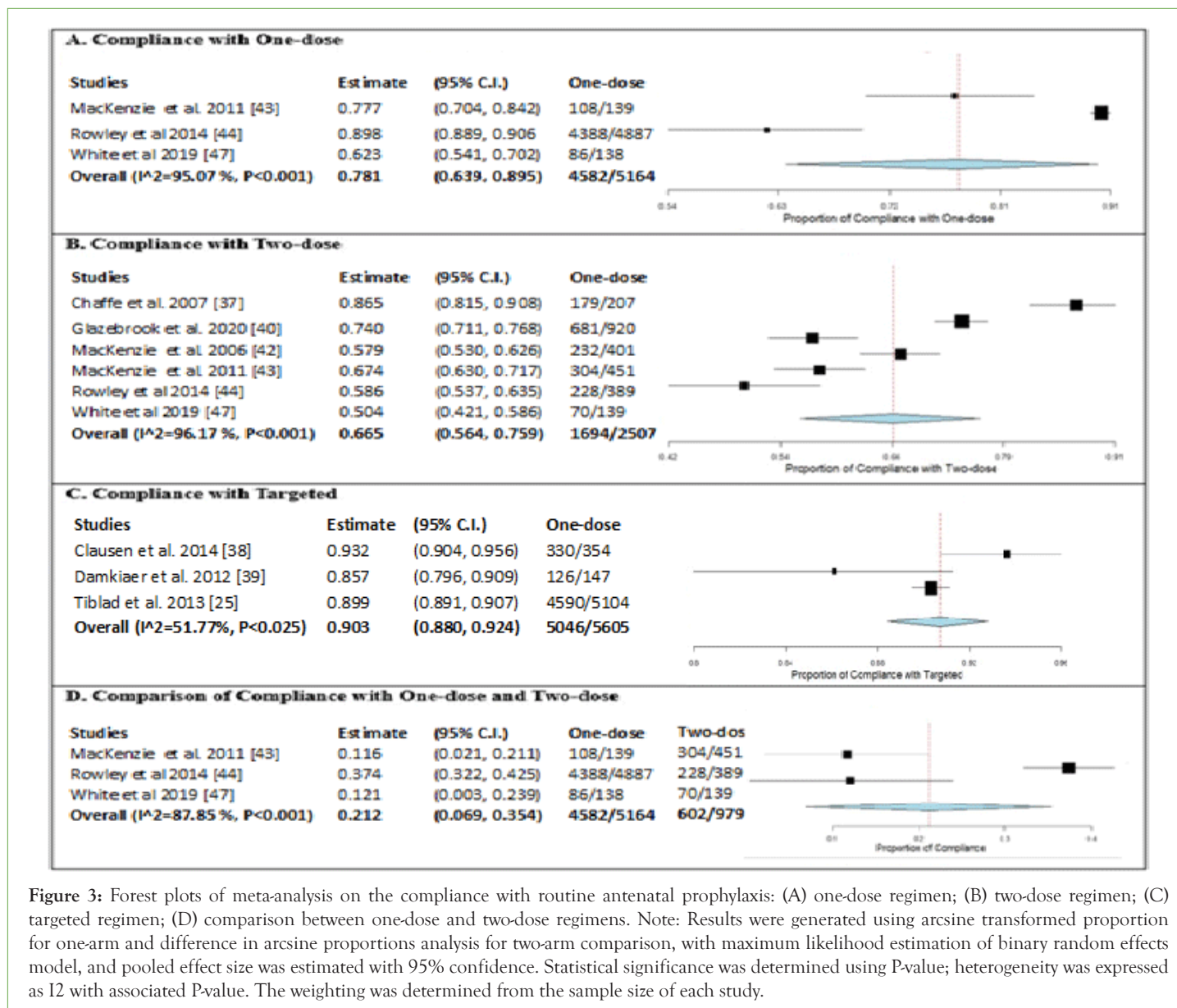
### Meta-analysis of RhD alloimmunisation after delivery

Meta-analysis on RhD alloimmunisation after delivery was performed for one-dose and two-dose regimens, with results presented in forest plots. The one-arm proportion analyses were statistically significant for one-dose (estimate 0.002, 95% CI [0.000, 0.005], P<0.001), and two-dose regimens (estimate 0.003, 95% CI [0.002, 0.005], P<0.001). The overall results showed that 0.13% of women were immunised after one-dose regimen, while 0.33% of women were immunised after two-dose regimen. The results found no heterogeneity for one-dose (I<sup>2</sup>=0%, P=0.977) and two-dose regimen (I<sup>2</sup>=0%, P=0.901), indicating no variability in data for studies included in the meta-analyses. Overall, the results suggested that there was a slightly higher rate of RhD alloimmunisation after two-dose regimen than one-dose regimen.

### Meta-analysis of compliance

A meta-analysis was performed on compliance with one-dose, two-dose, and targeted regimens, with results presented in forest plot (Figure

3). The one-arm proportion analyses were statistically significant for one-dose (estimate 0.781, 95% CI [0.639, 0.895], P<0.001), two-dose (estimate 0.665, 95% CI [0.564, 0.759], P<0.001), and targeted regimens (estimate 0.903, 95% CI [0.880, 0.924], P<0.001), with total compliance of 88.73%, 67.57% and 90.01%, respectively (Figures 3A-3C). The results were highly heterogeneous for one-dose (I<sup>2</sup>=95.07%, P<0.001) and two-dose regimens (I<sup>2</sup>=96.17%, P<0.001) while moderately heterogeneous for targeted regimen (I<sup>2</sup>=51.77%, P=0.025), indicating variability in data for studies included in the meta-analyses. Overall, the results suggested that the targeted regimen has the highest compliance, followed by one-dose and two-dose regimens. In Figure 3D, a two-arm proportion analysis was performed to compare the compliance of one-dose and two-dose regimens. The results of three included studies demonstrated a significant difference between the compliance of one-dose and two-dose regimens (estimate 0.212, 95% CI [0.069, 0.354], P=0.004), where one-dose regimen has higher compliance than two-dose regimen (88.73% compared to 61.49%). The heterogeneity was substantial (I<sup>2</sup>=87.85%, P<0.001).



**Figure 3:** Forest plots of meta-analysis on the compliance with routine antenatal prophylaxis: (A) one-dose regimen; (B) two-dose regimen; (C) targeted regimen; (D) comparison between one-dose and two-dose regimens. Note: Results were generated using arcsine transformed proportion for one-arm and difference in arcsine proportions analysis for two-arm comparison, with maximum likelihood estimation of binary random effects model, and pooled effect size was estimated with 95% confidence. Statistical significance was determined using P-value; heterogeneity was expressed as  $I^2$  with associated P-value. The weighting was determined from the sample size of each study.

## DISCUSSION

### Effectiveness of routine antenatal prophylaxis

The results showed that 18.35% of women after one-dose regimen and 24.57% of women after two-dose had detectable anti-D at delivery, suggesting two-dose regimen was relatively more effective than one-dose regimen. Theoretically, the half-life of circulating anti-D was 17-21 days [49,50]. Thus, the residual dose by calculation would be higher after 12 weeks of administering the primary dose for two-dose regimen than one-dose regimen. With a half-life of 21 days, the calculated residual dose after 12 weeks was 18.75  $\mu\text{g}$  after a single dose of 300  $\mu\text{g}$  (1500 IU), and 31.75  $\mu\text{g}$  after two doses of 100  $\mu\text{g}$  (500 IU) at 28 and 34 weeks' gestation [51]. Therefore, two-dose regimen proposed a theoretical advantage over one-dose regimen as more women were at risk of potential RhD sensitisation after one-dose regimen due to reduced protection from RhIG.

The World Health Organisation (WHO) suggested a minimal dose of 25  $\mu\text{g}$  (100 IU) for the prevention of RhD sensitisation with 1 mL of RhD-positive foetal red cells or approximately 2 mL of foetal whole blood [52]. However, it was believed that an unprovoked transplacental haemorrhage during pregnancy unlikely to be as large as the estimated

FMH of 1 mL foetal red cells at delivery [51]. Hence, both regimens were considered as effective. In addition, both regimens showed approximately 80% of women with no detectable anti-D. Rudensky et al. hypothesised that the non-detectable of passive anti-D was a result of RhIG binding to foetal red cells leading to either the clearance from maternal circulation before stimulating maternal immune system or presenting foetal red cells in a non-stimulatory form [45]. Therefore, the lack of detectable anti-D may reflect the effectiveness of antenatal RhIG.

Interestingly, the results showed more women were RhD alloimmunised after two-dose regimen compared to one-dose regimen. If more women had detectable anti-D at delivery after two-dose regimen, the RhD alloimmunisation rate would be expected to be lower with two-dose regimen given better protection from RhIG. Thus, suggesting other probable contributing factors leading to compromising efficacy of RhIG. A lower compliance rate with two-dose regimen may have resulted in an increased incidence of RhD alloimmunisation. Missing second dose and incorrect timing for administering second dose may lead to a lower concentration of RhIG in maternal circulation resulting in inadequate protection against RhD sensitisation until delivery.

Despite receiving antenatal according to recommended guidelines, RhD alloimmunisation was still prevalent indicating failure of routine



antenatal RhIG. Davies et al. found that women who delivered after 40 weeks' gestation had 1.5-3.5 times more risk of RhD sensitisation post administration of antenatal RhIG at 28 weeks' gestation [8]. In addition, some studies observed a shorter half-life of RhIG in majority of pregnant women where a decline in detectable anti-D happened before 40 weeks' gestation [41,42,50,51,53]. Other proposed explanations for the failure of antenatal RhIG included inadequate uptake of RhIG after intramuscular administration and failure to recognise silent massive FMH [42,50,51,53].

### Compliance of routine antenatal prophylaxis

The results revealed suboptimal compliance with all three regimens. There were several reasons for declining routine antenatal prophylaxis program including fear of infection, religious reason, concern about additives, partner was known to be RhD negative, last planned pregnancy, and no documented reason [37,40,43]. As for non-compliant with guidelines, the most common reason may be due to poor documentation of RhIG administration [39,40,42,44]. Appropriate RhIG administration documentation is critical for traceability and accessibility of detailed patient information by laboratories and clinical staff to ensure RhD-negative women have been properly managed [40]. Another reason for non-compliant for all three regimens was incorrect timing [37,40,42-44,47]. Administration of RhIG before recommended at 28 weeks can lead to lower level of passive anti-D in circulation close to delivery, while administration of RhIG too late can lead to potential sensitisation by silent FMH [44].

Glazebrook et al. demonstrated that lack of appropriate education on routine antenatal prophylaxis by responsible midwives and clinicians to RhD-negative pregnant women for making an informed decision [40]. It is significant that RhD-negative women understand the risk associated with being RhD negative, the significance of RhD alloimmunisation, and possibly affecting subsequent pregnancies. White et al. concluded that poor compliance for both one-dose and two-dose regimens were a result of systematic errors [47].

The compliance rate for targeted regimen was probably compromised as studies were conducted in the first few months after implementation and sources of information regarding antenatal NIPT and targeted antenatal RhIG may not be well known [38,39]. Improvement in compliance was observed over time [25]. However, targeted regimen had the highest compliance rate among the three regimens. The possible explanation could be that knowing the RHD status of foetus served as a motivating factor for receiving the antenatal RhIG at 28 weeks' gestation.

The results from meta-analysis revealed that one-dose regimen had a higher compliance than two-dose regimen (95% CI 0.069, 0.354,  $P < 0.01$ ). Such results were expected as the second dose of a two-dose regimen increased the chances of non-compliant due to several reasons. The most rational reason that women were not as compliant with two-dose regimen is women experienced early labour or potential sensitising events before second dose [44,47]. However, there are other concerning reasons including forgetting to attend appointment for second dose, lack of continuity of care, and underestimating the consequence of omission of second dose. White et al. found that missing a dose and receiving a dose late were common in two-dose regimen than one-dose regimen [47]. Missing of second dose may result in insufficient protection against sensitisation and increases the risk of RhD alloimmunisation in women which can be avoided. The importance of routine antenatal RhIG at 28 and 34 weeks' gestation should be emphasised thus encouraging RhD-negative women to

attend the designed appointments on time to minimise potential risk for RhD alloimmunisation.

### Limitations

The major limitation of this study is most included studies were retrospective in nature, which may introduce bias and/or confounding leading to controversies about the validity of the results. The information on potential confounding factors may be absent as the data were collected in the past. Differential losses to follow up leading to bias which may compromise the validity of the study. Therefore, the quality of historical records is dependent on the accuracy and completeness of the available data recorded in the healthcare databases. Moreover, the difference in methodologies and dosing and substantial heterogeneity of the included studies may limit the certainty of the overall effect size of the meta-analysis. Therefore, one should be cautious when interpreting the results. In addition, this study only evaluated compliance with targeted regimen with a broad definition. Therefore, further study is required to investigate full compliance with targeted regimen by considering the uptake of NIPT by women and targeted antenatal RhIG given at the correct gestational age. This is critical as eligible pregnant women who declined or missed NIPT will not be identified for antenatal RhIG.

### CONCLUSION

Overall, both one-dose and two-dose regimens were equally effective although slight differences in detectable anti-D and RhD alloimmunisation were observed. However, the one-dose regimen had higher compliance than the two-dose regimen, thus one-dose regimen may have performed better than two-dose regimen. Given that the current targeted regimen uses a single dose of antenatal prophylaxis which is similar to the one-dose regimen, one could conclude that the targeted regimen is the best strategy for routine antenatal prophylaxis with the highest compliance. The targeted regimen can avoid approximately 40% unnecessary administration of antenatal RhIG and conserve RhIG for pregnant women who are at risk of RhD sensitisation [32,54]. Several studies performed cost analysis on targeted regimen and suggested that targeted regimen might not be sufficiently cost effective to be implemented routinely [55,56]. While others suggested that targeted regimen enables cost saving from omitting cord blood serology, and reduced use of anti-D products and its associated risks [54,57]. The risks and benefits should be carefully assessed before the implementation of targeted regimen. However, the scarcity of RhIG may encourage the implementation of targeted regimen by many countries in the near future.

### CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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