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# A Clinical Practice Guideline for the Management of Pregnancy Alloimmunized to Red Blood Cell Antigens

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## Abstract

**IMPORTANCE** Red blood cell alloimmunization is typically associated with the transplacental transfer of incompatible fetal red blood cells into maternal circulation. Subsequent pregnancies can be affected by fetal anemia, hydrops fetalis, and perinatal death. Most cases of Rhesus D (RhD) alloimmunization due to pregnancy can be prevented by the proper administration of Rhesus immune globulin. However, an emerging practice of using low-titer, O, RhD-positive whole blood (LTOWB) in cases of life-threatening hemorrhage has the potential to increase the exposure of the female population to a new source of incompatible red blood cells.

**OBJECTIVE** To establish recommendations for the management of the red blood cell alloimmunized pregnancy.

**EVIDENCE** Four working groups were assembled that included experts in (1) trauma and transfusion medicine, (2) hematology, (3) maternal-fetal medicine/obstetrics, and (4) neonatology. Patient stakeholders and ethics representatives were included in each working group. The patient/problem, intervention, comparison, outcome (PICO) framework was used to identify key clinical knowledge gaps. Library scientists at Johns Hopkins University performed systematic reviews and meta-analyses on these topics and provided final reports to the working groups. All 4 working groups participated in a Delphi process to refine recommendations and practice points for each PICO question that reflected consideration of the following factors: balance of benefits and harms; certainty of evidence; values and preferences; resource use and costs; ethics; equity; and feasibility.

**FINDINGS** Seven clinical recommendations and 32 practice points were developed by the maternal-fetal medicine/obstetrics working group. Recommendations included the following: use of cell-free fetal DNA to identify the at-risk fetus early in pregnancy, followed by immunomodulation with intravenous immune globulin (IVIG) in select cases; the implementation of middle cerebral artery peak systolic velocity Doppler measurements to detect fetal anemia earlier in pregnancy; the use of IVIG in patients with a documented antigen-positive fetus with a history of either fetal anemia or a fetal loss due to hemolytic disease of the fetus and newborn before 24 weeks' gestational age in a previous pregnancy; the continuation of intrauterine transfusion therapy until the end of the 35th week of pregnancy; and prolonging gestational age to between 37 weeks 0 days and 38 weeks 6 days before proceeding to delivery.

**CONCLUSIONS AND RELEVANCE** These recommendations provide an updated approach to the management of red blood cell alloimmunized pregnancies. The lack of high-quality evidence limits the strength of the recommendations but points to the need for a standardized approach to this rare disease.

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+ Supplemental content

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## Introduction

Exposure to incompatible red blood cell antigens typically occurs in association with pregnancy. Although alloimmunization to the Rhesus c (Rhc), RhE, and Kell antigens are associated with severe fetal anemia through the transplacental passage of the maternal antibodies, RhD is the most antigenic and accounts for the majority of cases of hemolytic disease of the fetus and newborn (HDFN).[1] Rhesus immune globulin administered at 28 weeks of gestation and again after delivery has proven effective in preventing RhD alloimmunization in the majority of cases, although failures still occur due to inadvertent omissions.[2]

Recently, there has been a resurgence of interest in administrating whole blood to patients with life-threatening bleeding based on both military and civilian data suggesting improved survival.[3] Due to the shortage of RhD-negative red blood cell units, civilian emergency medical services and in-hospital blood banks have implemented the use of low-titer, O+ whole blood (LTOWB) for the treatment of severe traumatic bleeding.[4] Since rapid blood typing is not available in these situations, D-negative female trauma patients are at risk for exposure to D-positive red blood cells. In such circumstances, alloimmunization with anti-D antibodies occurs in approximately 20% of these patients.[5]

In 2024, the Trauma Hemostasis and Oxygenation Research (THOR) Network Foundation, the US Department of Defense (DOD), and the Allo Hope Foundation launched an effort to develop clinical practice guidelines in the management of D-negative female children and adolescent girls and women aged 15 to 49 years who were at risk for red blood cell alloimmunization as a result of exposure to LTOWB. The maternal-fetal medicine/obstetrics working group was tasked to develop up-to-date clinical recommendations for the management of red blood cell alloimmunized pregnancies.

## Methods

These guidelines follow international standards for guideline development and have been reported in accordance with the Appraisal of Guidelines for Research and Evaluation II (AGREE II) reporting checklist.[6,7] Detailed methods are provided in eMethods in the Supplement. The guideline panel included expertise in obstetrics and maternal fetal medicine; the working group also included a bioethicist and patient advocates. Members of the trauma and transfusion, hematology, maternal-fetal medicine/obstetrics, and neonatology working groups are listed in eTable 1 in the Supplement.

The maternal-fetal medicine/obstetrics working group used the patient/problem, intervention, comparison, and outcome (PICO) framework to identify key clinical knowledge gaps (**Box**). A team at Johns Hopkins University was commissioned to conduct systematic reviews. Protocols were registered on PROSPERO for the 6 systematic reviews conducted for this guideline (CRD42024512268, CRD42024512256, CRD42024512261, CRD42024512274, CRD42024512275, and CRD42024512271). One of the 6 systematic reviews was conducted as an update of a prior review.[8] Searches were conducted in February 2024 using PubMed and Embase and, for questions related to interventions, the Cochrane Central Register of Controlled Trials (CENTRAL). Trials and observational studies were considered. The certainty of the evidence for critical outcomes identified a priori was assessed using Grading of Recommendations Assessment, Development and Evaluation (GRADE).[9] PICO Portal was used to sort citations. Details on systematic review methods, including the inclusion and exclusion criteria for each research question, are presented in eTable 2 in the Supplement.

The working group presented draft recommendations at a public meeting on November 19, 2024. Revised recommendations were voted on by all working group members in a closed guideline meeting on November 20, 2024. Consensus was defined prior to voting as at least 75% agreement. Voting was conducted online using Poll Everywhere. If a recommendation did not achieve consensus after 3 rounds of voting, then that recommendation was considered to have not reached consensus.

During the Delphi process, all 4 working groups voted on the 7 recommendations and 32 practice points developed by the maternal-fetal medicine and obstetrics working group. No recommendation or practice point was rejected based on a lack of consensus. The final recommendations and the rationale to support them were provided for public comment from patients contacted via the Allo Hope Foundation and from experts recruited through the associations and societies that were represented within the work groups.

## Results

A total of 15 individuals (6 [40%] women) participants. The 12 clinicians in the maternal-fetal medicine/obstetrics working group had a mean (SD) of 21.2 (8.7) years of practice.

### Determination of Fetal Red Blood Cell Antigen Status

**Evidence-Based Recommendation**

We recommend the use of cell-free fetal DNA (cffDNA) to accurately determine the fetal red blood cell antigen status drawn after 10 weeks' gestational age in pregnancies complicated by RhD, RhC, Rhc, RhE, Kell, or Fy[a] alloimmunization (**Table**). This recommendation reached 92.5% agreement.

Our systematic review yielded 49 studies[8,10-65] (**Figure 1**) in 50 publications that evaluated the diagnostic accuracy of cffDNA for fetal antigen typing, 21 of which (reported in 22 publications[37-40,42,43,45,46,49-64]) were previously evaluated in a 2016 review by Mackie et al.[8] Included studies; number of index tests; true-positive, false-positive, true-negative, false-negative, and inconclusive results; and sensitivity and specificity are reported in eTable 3 in the Supplement. When performed at an appropriate gestational age, cffDNA testing for fetal antigen typing had high sensitivity and specificity for determining fetal antigen status when compared with neonatal testing, amniocentesis, or chorionic villus sampling (pooled sensitivity, 100%; 95% CI, 99%-100%; certainty

**Box. PICO Questions for the Management of Red Blood Cell Alloimmunized Pregnancies**

**Diagnostic Accuracy, Risks, and Benefits of Cell-Free Fetal DNA**
- What is the diagnostic accuracy of cell-free fetal DNA in determining the fetal red blood cell antigen status?
- What are the risks and benefits of using cell-free fetal DNA vs amniocentesis or chorion villus biopsy to determine the fetal red blood cell antigen status?

**Critical Titer to Institute MCA-PSV Doppler Studies in Kell Alloimmunized Pregnancies**
- What are the benefits and harms of using a critical titer of less than 4 in the Kell alloimmunized pregnancy?

**Timing of MCA-PSV Doppler**
- Among women with a current or previous pregnancy with red blood cell antibodies known to cause HDFN, what are the benefits and harms of starting weekly serial MCA-PSV Doppler measurements at 18 weeks' gestation vs at 16 weeks' gestation?

**Benefits and Harms of Immunomodulation**
- What are the benefits and harms of not using immunomodulation (intravenous immune globulin and/or plasmapheresis) in patients with a history of early-onset severe red blood cell alloimmunization, defined as evidence of fetal severe anemia and/or fetal loss due to HDFN before 24 weeks' gestation in a prior pregnancy?
- What are the benefits and harms of not using immunomodulation in patients with an alloimmunized pregnancy (ie, patients with severe red blood cell alloimmunization with a proven or suspected antigen-positive fetus and a titer for anti-D ≥512 or a titer for anti-Kell ≥64 regardless of prior pregnancy history)?

**Timing of Stopping Intrauterine Transfusions**
- Among women with a current or previous pregnancy with red blood cell antibodies known to cause HDFN who have received at least 1 intrauterine transfusion, what are the benefits and harms of stopping intrauterine transfusions prior to 35 weeks' gestation vs stopping at 35 weeks' gestation or later?

**Timing of Delivery**
- Among women with a current or previous pregnancy with red blood cell antibodies known to cause HDFN and an antigen-positive fetus who have not received an intrauterine transfusion, what are the benefits and harms of delivery prior to 37 to 38 weeks' gestation?

Abbreviations: HDFN, hemolytic disease of the fetus/newborn; MCA-PSV, middle cerebral artery peak systolic velocity.

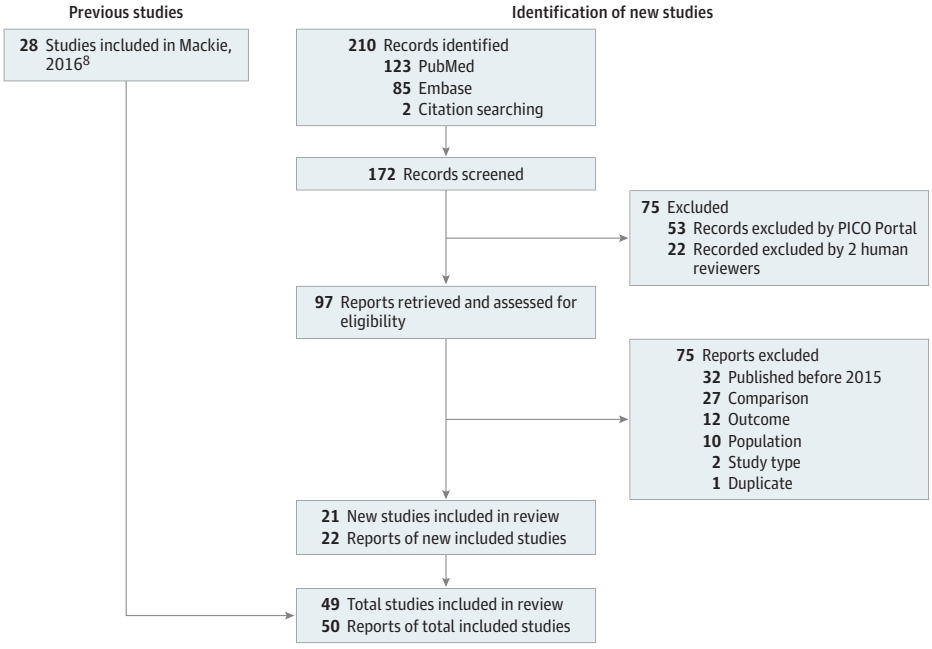
of evidence [COE], moderate; pooled specificity, 98%; 95% CI, 97%-99%; COE, moderate) (**Figures 2** and **3**). Since some cffDNA studies compared its accuracy to genotyping by amniocentesis or chorion villus biopsy instead of fetal or neonatal serology, the accuracy of testing may have been overestimated. These results are consistent with Mackie et al,[8] which reported a pooled sensitivity of 99.3% (95% CI, 98.2%-99.7%) and pooled specificity of 98.4% (95% CI, 96.4%-99.3%).[8] Since the conduct of the current systematic review, an additional study by Rego and colleagues[10] compared 465 cffDNA samples including 143 for Kell, 124 for RhE, 60 for RhC, 50 for Fy$^a$, 47 for Rhc, and 41 for RhD, with 100% concordance with neonatal genotyping results.

**Table. Recommendations for the Management of the Red Blood Cell Alloimmunized Pregnancy**

| Recommendation | Agreement, % | Confidence of in quality of evidence |
|---|---|---|
| 1: We recommend the use of maternal cell-free fetal DNA to accurately determine the fetal red blood cell antigen status drawn after 10 weeks' GA in pregnancies complicated by RhD, RhC, Rhc, RhE, Kell or Fy$^a$ alloimmunization. | 92.5% | Moderate |
| 2a: We recommend the use of IVIG in patients with a documented antigen-positive fetus with a history of either fetal anemia or a fetal loss due to HDFN before 24 weeks' GA in a previous pregnancy. | 100% | Very low |
| 2b: We suggest the use of IVIG in patients in a pregnancy with a documented antigen-positive fetus and a titer for anti-D of ≥512 or a titer for Kell ≥64 regardless of prior pregnancy history. | 100% | Very low |
| 3: We recommend that surveillance with MCA-PSV Doppler measurements be initiated when the maternal Kell titer is 4 or greater or there is a history of an affected fetus or neonate in an antecedent pregnancy. | 97.5% | Very low |
| 4: We recommend that MCA-PSV Doppler measurements be initiated weekly by 16 weeks' GA in patients with red blood cell antibodies associated with HDFN when there is an antigen-positive fetus or antigen-unknown fetus once a critical titer threshold has been reached. A critical titer is defined as 16 or greater for most antibodies and 4 or greater for anti-Kell. | 92.7% | Very low |
| 5: We recommend for women undergoing intrauterine transfusions in pregnancy for the treatment of HDFN, intrauterine transfusions should be continued until the end of the 35th week of gestation unless there are technical limitations to undertaking the procedure. | 97.6% | Very low |
| 6: We recommend that in women with a current or previous pregnancy with red blood cell antibodies known to cause HDFN regardless of titer who have not received an intrauterine transfusion, delivery should occur between 37 weeks 0 days' to 38 weeks 6 days' GA. | 92.7% | Very low |

Abbreviations: GA, gestational age; HDFN, hemolytic disease of the fetus/newborn; IVIG, intravenous immune globulin; MCA-PSV, middle cerebral artery peak systolic velocity; Rh, Rhesus.

**Figure 1. Flow Diagram for Literature Review of Cell-Free Fetal DNA for Fetal Antigen Typing**

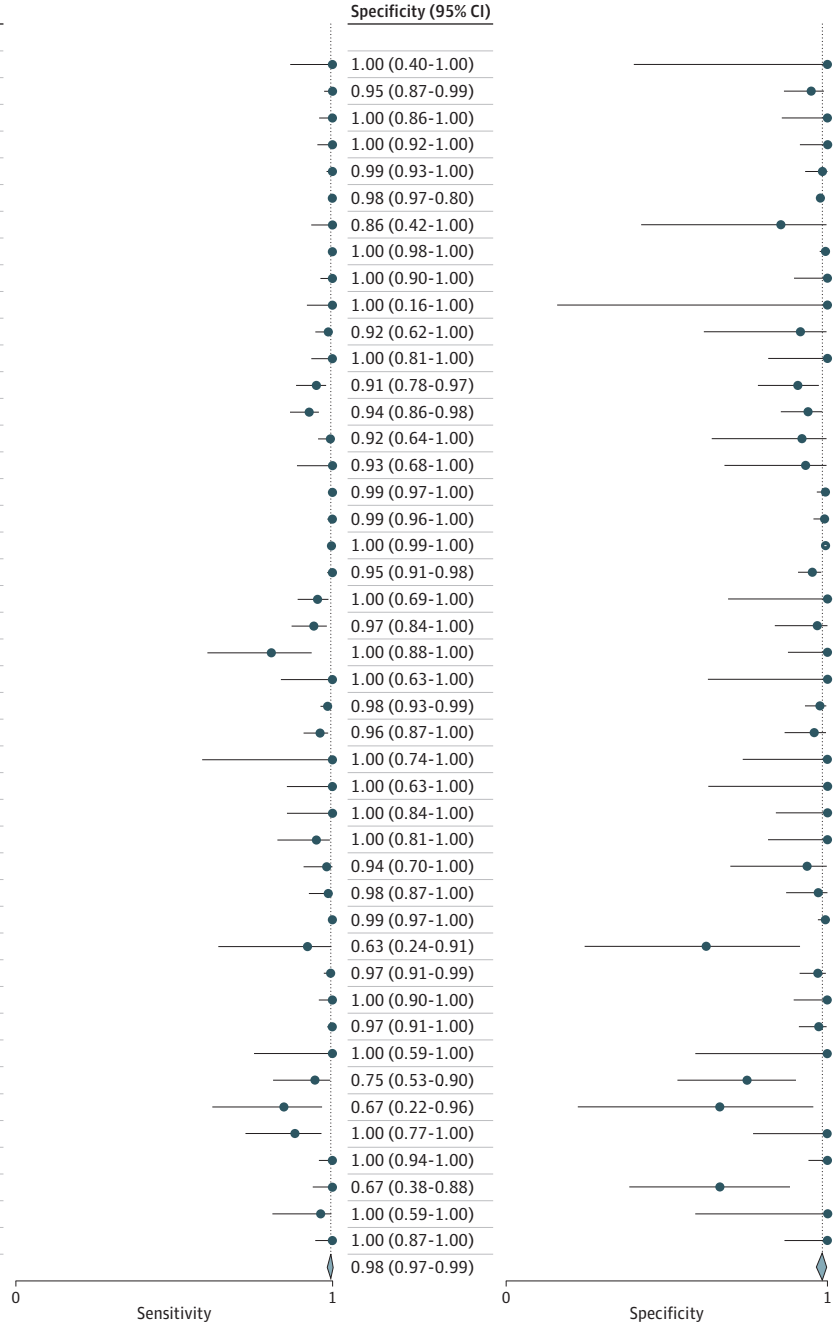


### Rationale

No studies were identified that evaluated the risks and benefits of using cffDNA; however, the utility of this diagnostic medium was evaluated within the voting committee considering patient preference and health care burden. cffDNA was favored by patients and clinicians as an alternative to amniocentesis as, unlike amniocentesis, it is noninvasive and does not carry a risk of ruptured membranes, pregnancy loss, or enhancing maternal antibody titer.[11] cffDNA was also preferred over paternal antigen phenotyping and zygosity testing because it eliminates the risk of inaccurate results from uncertain paternity, avoids the delay in pregnancy management as an intermediate step in determining fetal risks, and eliminates human error of inadvertently ordering paternal antibody

Figure 2. Pooled Sensitivity and Specificity of Cell-Free Fetal DNA for Fetal Antigen Typing, Rhesus D

| Source | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|
| Rhesus D | | |
| Ahmadi et al,[15] 2016 | 1.00 (0.87-1.00) | 1.00 (0.40-1.00) |
| Bingulac-Popovic et al,[16] 2021 | 1.00 (0.97-1.00) | 0.95 (0.87-0.99) |
| Blanco et al,[17] 2018 | 1.00 (0.96-1.00) | 1.00 (0.86-1.00) |
| Blomme et al,[18] 2022 | 1.00 (0.95-1.00) | 1.00 (0.92-1.00) |
| Boggione et al,[19] 2017 | 1.00 (0.98-1.00) | 0.99 (0.93-1.00) |
| de Haas et al,[20] 2016 | 1.00 (1.00-1.00) | 0.98 (0.97-0.80) |
| Duan et al,[21] 2023 | 1.00 (0.94-1.00) | 0.86 (0.42-1.00) |
| Hyland et al,[22] 2017 | 1.00 (0.99-1.00) | 1.00 (0.98-1.00) |
| Londero et al,[23] 2019 | 1.00 (0.96-1.00) | 1.00 (0.90-1.00) |
| Moezzi et al,[24] 2016 | 1.00 (0.92-1.00) | 1.00 (0.16-1.00) |
| Niguse et al,[25] 2022 | 0.99 (0.95-1.00) | 0.92 (0.62-1.00) |
| Papasavva et al,[26] 2016 | 1.00 (0.93-1.00) | 1.00 (0.81-1.00) |
| Pazourkova et al,[27]2021 | 0.95 (0.89-0.98) | 0.91 (0.78-0.97) |
| Picchiassi et al,[28] 2015 | 0.93 (0.87-0.97) | 0.94 (0.86-0.98) |
| Rather et al,[29] 2019 | 0.99 (0.96-1.00) | 0.92 (0.64-1.00) |
| Sillence et al,[30] 2015 | 1.00 (0.89-1.00) | 0.93 (0.68-1.00) |
| Soothill et al,[31] 2015 | 1.00 (0.99-1.00) | 0.99 (0.97-1.00) |
| Sorensen et al,[32] 2018 | 1.00 (0.98-1.00) | 0.99 (0.96-1.00) |
| Uzenel et al,[33] 2022 | 1.00 (1.00-1.00) | 1.00 (0.99-1.00) |
| Vivanti et al,[34] 2016 | 1.00 (0.99-1.00) | 0.95 (0.91-0.98) |
| Yasa et al,[35] 2020 | 0.96 (0.89-0.99) | 1.00 (0.69-1.00) |
| Acharqui et al,[37] 2011 | 0.94 (0.87-0.98) | 0.97 (0.84-1.00) |
| Al-Yatama et al,[38] 2007 | 0.81 (0.61-0.93) | 1.00 (0.88-1.00) |
| Aykut et al,[39] 2010 | 1.00 (0.84-1.00) | 1.00 (0.63-1.00) |
| Bombard et al,[40] 2011 | 0.99 (0.96-1.00) | 0.98 (0.93-0.99) |
| Grill et al,[42] 2009 | 0.96 (0.91-0.99) | 0.96 (0.87-1.00) |
| Gunel et al,[43] 2010 | 1.00 (0.59-1.00) | 1.00 (0.74-1.00) |
| Han et al,[45] 2012 | 1.00 (0.86-1.00) | 1.00 (0.63-1.00) |
| Hromadnikova et al,[46] 2005 | 1.00 (0.86-1.00) | 1.00 (0.84-1.00) |
| Lo et al,[49] 1998 | 0.95 (0.83-0.99) | 1.00 (0.81-1.00) |
| Machado et al,[50] 2006 | 0.98 (0.91-1.00) | 0.94 (0.70-1.00) |
| Manzanares et al,[51] 2013 | 0.99 (0.93-1.00) | 0.98 (0.87-1.00) |
| Minon et al,[52] 2008 | 1.00 (0.99-1.00) | 0.99 (0.97-1.00) |
| Mohammed et al,[53] 2010 | 0.92 (0.64-1.00) | 0.63 (0.24-0.91) |
| Moise et al,[54] 2013 | 1.00 (0.98-1.00) | 0.97 (0.91-0.99) |
| Polin et al,[55] 2013 | 1.00 (0.96-1.00) | 1.00 (0.90-1.00) |
| Rouillac-Le Sciellour et al,[56] 2007 | 1.00 (0.98-1.00) | 0.97 (0.91-1.00) |
| Sbarsi et al,[57] 2012 | 1.00 (0.75-1.00) | 1.00 (0.59-1.00) |
| Sesarini et al,[58] 2009 | 0.94 (0.81-0.99) | 0.75 (0.53-0.90) |
| Siva et al,[59] 2003 | 0.85 (0.62-0.97) | 0.67 (0.22-0.96) |
| Turner et al,[60] 2003 | 0.88 (0.73-0.97) | 1.00 (0.77-1.00) |
| Tynan et al,[61] 2011 | 1.00 (0.96-1.00) | 1.00 (0.94-1.00) |
| Wang et al,[62] 2009 | 1.00 (0.94-1.00) | 0.67 (0.38-0.88) |
| Zhong et al,[63] 2001 | 0.96 (0.81-1.00) | 1.00 (0.59-1.00) |
| Zhou et al,[64] 2005 | 1.00 (0.95-1.00) | 1.00 (0.87-1.00) |
| Summary | 1.00 (0.99-1.00) | 0.98 (0.97-0.99) |



screens (rather than antigen zygosity testing). cffDNA was also favored over monitoring with middle cerebral artery (MCA) Doppler ultrasonography, which, in the case of an antigen-negative fetus, results in unnecessary costs and health care burden. Moreover, it creates a risk of false positive MCA peak systolic velocity (MCA-PSV) Doppler findings, leading to avoidable amniocentesis for fetal genotyping.[12] Widespread implementation of cffDNA testing should be accompanied by ethical safeguards related to genetic privacy and data governance, such as utilizing tests specifically targeted to red blood cell antigen genotyping rather than broader genomic panels or sequencing-based methods. Four practice points associated with this recommendation were developed and approved (eTable 4 in the Supplement).

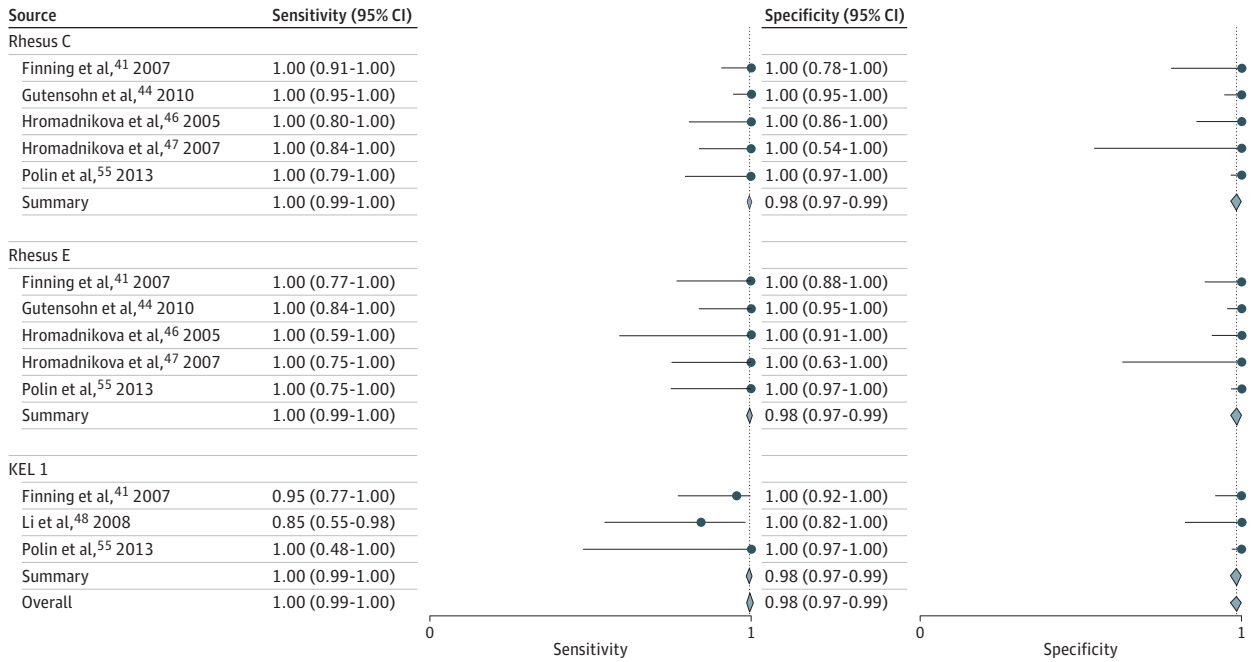
## Use of Intravenous Immune Globulin

### Evidence-Based Recommendation 2a: Patients With History of Fetal Anemia or Fetal Loss Due to HDFN

We recommend the use of intravenous immune globulin (IVIG) in patients with a documented antigen-positive fetus and a history of either fetal anemia or a fetal loss due to HDFN before 24 weeks' gestation age in a previous pregnancy (Table). This recommendation achieved 100% agreement.

Our systematic review identified a total of 7 cohort studies[66-72] evaluating the benefits and harms of immunomodulation. The range of mean gestational ages at first intrauterine transfusion (IUT) among those who received immunomodulation was 20.67 to 28.70 weeks compared with 20.43 to 24.00 weeks among those who did not receive immunomodulation; COE was very low. Subsequent to our review, Mustafa et al[73] reported an individual patient data meta-analysis of 97 cases and 97 controls. Gestational age at first IUT improved with IVIG treatment by 3.19 weeks (95% CI, 1.28 to 5.05 weeks; IRR, 1.32; 95% credible interval, 0.08 to 2.50). In our initial review, mean fetal hemoglobin at first IUT among those who received immunomodulation ranged between 5.40 and 6.72 g/dL vs 3.39 to 6.70 g/dL in those that did not receive IVIG (to convert hemoglobin to grams per liter, multiply by 10); COE was very low. Mustafa et al[73] found a similar mean fetal hemoglobin difference of 6.39 vs 4.29 g/dL (IRR, 2.09; 95% CI, 1.12 to 3.05) in those who did vs did not receive

Figure 3. Pooled Sensitivity and Specificity of Cell-Free Fetal DNA for Fetal Antigen Typing, Rhesus C, Rhesus E, and Kell 1



IVIG. The number of IUTs in our review indicated a mean range of 2.50 to 5.00 with immunomodulation vs 2.25 to 5.00 transfusions among those who did not receive immunomodulation; COE was very low. This is similar to the review by Mustafa et al,[73] who found an IRR of 0.41 (95% CI, −0.26 to 1.06). Finally, the pooled risk ratio for the overall perinatal survival was 1.07 (95% CI, 0.94 to 1.22); COE was very low. The review by Mustafa et al[73] found a more favorable rate of survival at birth when IVIG was used in pregnancy (86.6% vs 47.4%; IRR, 1.82; 95% CI, 1.30 to 2.61).

#### Rationale

Although the data suggest that the alloimmunized patient treated with IVIG will still require IUT therapy, more recent analysis suggests that the first IUT will be performed later in gestation and the fetus will have less severe anemia at the first procedure. Patients with a history of pregnancy treated with IUTs will on average require the onset of IUTs 3 weeks earlier in a subsequent gestation.[74] IUTs performed at less than 22 weeks' gestational age are associated with a higher rate of perinatal loss.[75] Our working group decided IVIG should be offered to a patient with a history of early-onset HDFN in an effort to delay the gestational age at the first IUT. Patient counseling regarding the risks of IVIG should be undertaken prior to its administration.

#### Expert Opinion Recommendation 2b: Among Patients With Documented Antigen-Positive Fetus

We suggest the use of IVIG in patients in a pregnancy with a documented antigen-positive fetus and an initial titer for anti-D of 512 or greater or an initial titer for Kell of 64 or greater regardless of prior pregnancy history (Table). This recommendation achieved 100% agreement.

#### Rationale

Although most published series of the treatment outcomes of IVIG involve patients with a history of earlonset HDFN, it is ethically appropriate to offer this same therapy to patients with very high antibody titers who are at increased risk for requiring IUTs. Denying access to a potentially beneficial therapy solely based on prior pregnancy history could be considered unjust, especially when the anticipated risks of early fetal anemia are high. Eight practice points associated with these 2 recommendations were developed and approved (eTable 4 in the Supplement).

## Surveillance With MCA-PSV Doppler

#### Evidence-Based Recommendation 3

We recommend that surveillance with MCA-PSV Doppler measurements be initiated when the maternal Kell titer is 4 or greater or when there is a history of an affected fetus or neonate in an antecedent pregnancy (Table). This recommendation achieved 97.5% agreement.

Our systematic review identified 1 study[76] that evaluated the diagnostic accuracy of Kell antibody titers in predicting the development of HDFN. Kell antibodies are believed to cause fetal anemia by both hemolysis and bone marrow suppression, potentially leading to moderate or severe anemia at lower antibody titers than is seen for other red blood cell antibodies that do not exert hypoproliferative effects upon fetal bone marrow.[77] Slootweg and colleagues[76] performed a retrospective cohort study in the Netherlands from 1999 to 2015, including 92 pregnancies (with 93 fetuses) with Kell antibodies and Kell-positive fetuses. More than half of these children (52%) required IUTs. The optimal cutoff titer for identifying cases at a high risk for severe HDFN was found to be 4, a titer with a specificity of 27% and a positive predictive value for fetal or neonatal transfusion of 64%. Overall perinatal survival rates were reported to be 100% among those with a titer of less than 4 and 96.8% among those with a titer of 4 or greater. In the past, some experts have proposed excluding the titer entirely from risk determination for HDFN, recommending initiation of MCA-PSV Doppler screening for anemia regardless of the titer.[78] Although one report[79] was identified in the literature of a hydropic fetus requiring an IUT at 19 weeks, 2 days' gestation despite

a Kell titer of only 2, this is believed to be a rare occurrence. Since the conduct of the current systematic review, Vlachodimitropoulou and coworkers[80] reviewed 7 series of pregnancy reports of Kell alloimmunization and suggested that an initial Kell titer of greater than 4 or a history of anti-Kell–mediated HDFN warrants initiation of fetal surveillance with MCA-PSV Doppler.

### Rationale

Although there is one case reported in the literature of significant HDFN occurring with an antibody titer of less than 4, this is believed to be an exceedingly rare occurrence. We must weigh this risk of significant disease in a low-titer pregnancy against the 12% false-positive rate of MCA-PSV screening for anemia, the occurrence of which can necessitate unnecessary invasive procedures with associated risks of pregnancy loss, preterm delivery, and fetal demise as well increased risk for maternal infection, bleeding, and injury to other organs.[81] The study group felt that exclusion of Kell titers in the calculation of risk determination for HDFN would not be judicious but instead recommended use of a lower titer (≤4) as a critical level to prompt further surveillance for fetal anemia. The selection of a titer threshold must balance ethical obligations to avoid harm (false reassurance from too-high thresholds) against the harms of overmonitoring and unnecessary procedures. Shared decision-making and transparent discussion of risks are critical. Two practice points associated with this recommendation were developed and approved (eTable 4 in the Supplement).

## Timing of MCA-PSV Doppler Measurements

### Expert Opinion Recommendation 4

We recommend that MCA-PSV Doppler measurements be initiated weekly by 16 weeks' gestation in patients with red blood cell antibodies associated with HDFN when there is an antigen-positive fetus or antigen-unknown fetus once a critical titer threshold has been reached. A critical titer is defined as 16 or greater for most antibodies and 4 or greater for anti-Kell (Table). This recommendation reached 92.7% agreement. Our systematic review did not identify any studies regarding the benefits and harms of initiating weekly MCA-PSV Doppler measurements at 18 vs 16 weeks' gestation among women with current or a history of HDFN.

### Rationale

Intravascular IUTs performed prior to 20 weeks' gestation are associated with a 3-fold increased risk of perinatal loss compared with those performed later in gestation.[82] However, intraperitoneal transfusions as early as 15 weeks' gestation have been reported as a successful method of bridging the need for IUTs until a later gestational age when fetal intravascular access is more easily attained.[83] As we can screen for moderate to severe anemia and intervene via IUTs as indicated starting at 16 weeks' gestation, it seems prudent to initiate monitoring for HDFN at this gestational age. Weekly monitoring allows for early diagnosis and prompt treatment of fetal anemia should it occur and minimizes the risks associated with false-negative results, thereby optimizing outcomes for fetuses at risk for this disease.[84] Three practice points associated with this recommendation were developed and approved (eTable 4 in the Supplement).

## Duration of IUT Therapy

### Expert Opinion Recommendation 5

For women undergoing IUTs in pregnancy for the treatment of HDFN, we recommend that IUTs be continued until the end of the 35th week of gestation unless there are technical limitations to undertaking the procedure (Table). This recommendation achieved 97.6% agreement. The systematic review did not identify any studies evaluating the timing of stopping IUT among adult women with a current or previous pregnancy with red blood cell antibodies known to cause HDFN who have received at least 1 IUT.

### Rationale

Prolongation of pregnancy can result in decreased neonatal morbidity and length of hospital stay, both of which could reduce overall medical costs. In a series of 937 IUTs performed for 334 fetuses, the risk of a procedure-related emergency cesarean delivery was 0.4% per procedure.[85] The risks for neonatal respiratory complications as well as infectious and neurologic morbidities have been shown to be highest in the late preterm infant at 35 weeks' gestational age, with a gradual decrease with advancing gestation until 39 weeks' gestational age.[86] Theoretically, in addition to a decrease in overall neonatal morbidity, a more advanced gestational age at delivery allows for the attainment of hepatic maturity with less need for neonatal exchange transfusion due to hyperbilirubinemia unresponsive to phototherapy. This hypothesis is supported by data from the DIONYSIS study of 1855 neonates with HDFN treated at 31 centers in 22 countries.[13] The incidence of exchange transfusion in neonates who previously received IUTs decreased from 38% when they were delivered at 34 weeks' gestational age to 17% with delivery after 37 weeks' gestation age. Neonatal exchange transfusions are associated with an 8-fold increased risk of proven sepsis, a 36-fold risk of leukopenia, a 31-fold risk of thrombocytopenia, a 27-fold risk of hypocalcemia and an 8-fold risk of hypernatremia.[87] These data are reflected in a Delphi survey of 107 experts in the management of HDFN from 25 countries that reached a 78% consensus that IUTs should be continued until a gestational age between 35 weeks 0 days' and 35 weeks 6 days' gestation.[88] In determining the timing of IUTs and delivery, clinicians should support informed maternal choice, grounded in a transparent understanding of the trade-offs between fetal and neonatal risks. Four practice points associated with this recommendation were developed and approved (eTable 4 in the Supplement).

## Delivery Timing

### Expert Opinion Recommendation 6

For women with a current or previous pregnancy with red blood cell antibodies known to cause HDFN, regardless of titer, who have not received an IUT, we recommend that delivery should occur between 37 weeks 0 days' to 38 weeks 6 days' gestational age (Table). This recommendation reached 92.7% agreement. Our systematic review did not identify any studies evaluating the timing of delivery in pregnancies complicated by a red blood cell antibody associated with HDFN regardless of titer and the need for IUT.

### Rationale

Current monitoring for fetal anemia involves the use of the MCA-PSV Doppler ultrasound. Due to a higher false-positive rate after 35 weeks' gestational age, some authors have advocated that the MCA-PSV Doppler is less reliable in late gestation.[12] Early-term delivery between gestational ages 37 weeks 0 days and 38 weeks 6 days could help to mitigate the risk of severe anemia-related complications, such as hydrops fetalis and stillbirth. Seven practice points associated with this recommendation were developed and approved (eTable 4 in the Supplement).

# Discussion

Previous US and British guidelines for the management of the red blood cell alloimmunized pregnancy were published more than a decade ago.[89,90] Since that time, several international surveys regarding the optimal management of these pregnancies have added new insight to inform a more standardized approach to the alloimmunized pregnancy.[13,88,91] In addition, new cffDNA assays using next-generation sequencing methods for determining fetal red blood cell antigen status have been introduced in the United States in the last 3 years.[10,92]

The maternal-fetal medicine/obstetrics working group of this THOR–Department of Defense–Allo Hope Foundation initiative sought to address these advances and new knowledge through the development of 6 major PICO questions (Box). Six recommendations (Table) and 32 accompanying practice points (eTable 4 in the Supplement) were developed and approved through an extensive

Delphi process. Although these recommendations were developed within a US framework, they would be applicable in other countries with available resources.

Our first recommendation regarding the use of cffDNA to determine fetal antigen status early in pregnancy was supported by extensive previous publications, mostly based on polymerase chain reaction methods in European studies. The 2 commercial assays now available in the US use narrowly targeted next-generation sequencing and are thought to be more robust for accuracy when various alterations for the *RHD* gene are detected in various ethnic groups.[93] The routine use of cffDNA was not addressed in the 2015 guideline from the Society for Maternal-Fetal Medicine (SMFM),[90] and our current recommendation suggests it can be used as early as 10 weeks' gestation, much earlier that the 16 to 20 weeks gestation suggested in 2014 by the Royal College of Obstetricians and Gynaecologists (RCOG).[89]

Our second recommendation on the use of IVIG in cases with a history of early onset of HDFN or severe red blood cell alloimmunization was not previously addressed in the guidelines from either SMFM or RCOG. The third recommendation from our working group suggested the use of a Kell titer value of 4 to define the threshold for beginning surveillance for fetal anemia with MCA-PSV Doppler. The SMFM guideline did not address this issue; however, the RCOG guideline suggested that severe anemia can occur even at low Kell titers. Recently the British Society of Hematology suggested that a Kell titer of 4 or greater warrants further investigation for fetal anemia.[94]

Recommendation 4 from our working group suggests starting fetal surveillance with MCA-PSV Doppler as early as 16 weeks' gestation once the fetal antigen status has been determined using cffDNA. Earlier SMFM guidance suggested initiating MCA-PSV Doppler measurements at 18 to 20 weeks gestation; RCOG did not address a specific gestational age to begin MCA-PSV Doppler. Finally, recommendations 5 and 6, regarding the continuation of IUTs until 35 weeks' gestational age and postponing delivery until a later gestational age than what has traditionally been proposed, are unique to our guideline.

### Limitations

The guideline development process for the management of rare diseases has several limitations. The limited amount of published evidence results in recommendations based on little or low-quality evidence. Ideally, recommendations should be based on clinical trials that demonstrate improved outcomes with certain interventions. However, because such evidence was limited, PICO questions were formulated to address gaps in current knowledge. Issues with risks, benefits, and disparity and ethical concerns were taken into account in the formation of our recommendations.

## Conclusions

Red blood cell alloimmunization and the subsequent fetal and newborn effects—including HDFN—can develop through multiple mechanisms of foreign red blood cell exposure and remains a global high-risk pregnancy issue. These clinical practice guidelines provide updated recommendations and practice points for the management of these pregnancies.

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**Additional Information:** PICO Portal was used from DATE to DATE to sort citations found in searches for the systematic reviews. The authors take responsibility for the integrity of the content generated.

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**SUPPLEMENT.**

**eMethods.**

**eTable 1.** List of Members of the Working Groups

**eTable 2.** Inclusion and Exclusion Criteria for Each Research Question

**eTable 3.** Diagnostic Accuracy of Free Fetal DNA for Fetal Antigen Typing, Stratified by Fetal Antigen Type

**eTable 4.** Good Practice Points and Rationales

**eReferences.**