Society for Maternal-Fetal Medicine (SMFM) Clinical Guideline #8: The fetus at risk for anemia—diagnosis and management

Society for Maternal-Fetal Medicine (SMFM); Giancarlo Mari, MD; Mary E. Norton, MD; Joanne Stone, MD; Vincenzo Berghella, MD; Anthony C. Sciscione, DO; Danielle Tate, MD; Mauro H. Schenone, MD

OBJECTIVE: We sought to provide evidence-based guidelines for the diagnosis and management of fetal anemia.

METHODS: A systematic literature review was performed using MEDLINE, PubMed, EMBASE, and the Cochrane Library. The search was restricted to English-language articles published from 1966 through May 2014. Priority was given to articles reporting original research, in particular randomized controlled trials, although review articles and commentaries were consulted. Abstracts of research presented at symposia and scientific conferences were not considered adequate for inclusion. Evidence reports and published guidelines were also reviewed, and additional studies were located by reviewing bibliographies of identified articles. GRADE (Grading of Recommendations Assessment, Development, and Evaluation) methodology was used for defining the strength of recommendations and rating the quality of evidence. Consistent with US Preventive Task Force guidelines, references were evaluated for quality based on the highest level of evidence.

RESULTS AND RECOMMENDATIONS: We recommend the following: (1) middle cerebral artery peak systolic velocity (MCA-PSV) measured by ultrasound Doppler interrogation be used as the primary technique to detect fetal anemia; (2) amniotic fluid delta OD450 not be used to diagnosis fetal anemia; (3) MCA-PSV assessment be reserved for those patients who are at risk of having an anemic fetus (proper technique for MCA-PSV evaluation includes assessment of the middle cerebral artery close to its origin, ideally at a zero degree angle without angle correction); (4) if a fetus is deemed at significant risk for severe fetal anemia (MCA greater than 1.5 multiples of the median or hydropic), fetal blood sampling be performed with preparation for an intrauterine transfusion, unless the pregnancy is at a gestational age when the risks associated with delivery are considered to be less than those associated with the procedure; (5) if a fetus is deemed at significant risk for severe fetal anemia, the patient be referred to a center with expertise in invasive fetal therapy; (6) MCA-PSV be considered to determine the timing of a second transfusion in fetuses with anemia, and, alternatively, a predicted decline in fetal hemoglobin may be used for timing the second procedure; and (7) pregnancies with a fetus at significant risk for fetal anemia be delivered at 37-38 weeks of gestation unless indications develop prior to this time.

Key words: amniocentesis, cordocentesis, Doppler, fetal anemia, fetal blood sampling, fetal complications, fetal hydrops, middle cerebral artery peak systolic velocity

Anemia continues to be an uncommon but life-threatening condition for the developing fetus. Red cell alloimmunization has historically been the most common cause of fetal anemia in the United States and in many other parts of the world. Other causes of fetal anemia include parvovirus infection and other less common conditions. This review describes the causes, surveillance options, and management strategies for the pregnancy at risk for fetal anemia.

From the Society for Maternal-Fetal Medicine Publications Committee, Washington, DC; the Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, The University of Tennessee Health Science Center, Memphis, TN (Dr Mari); the Division of Maternal-Fetal Medicine, University of California, San Francisco, San Francisco, CA (Dr Norton); Mount Sinai Hospital, New York, NY (Dr Stone); the Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA (Dr Berghella); the Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, Christina Care Health System, Wilmington, DE (Dr Sciscione); the Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, The University of Tennessee Health Science Center, Memphis, TN (Dr Tate); and the Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, The University of Tennessee Health Science Center, Memphis, TN (Dr Schenone).

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Corresponding author: Society for Maternal-Fetal Medicine: Publications Committee. pubs@smfm.org

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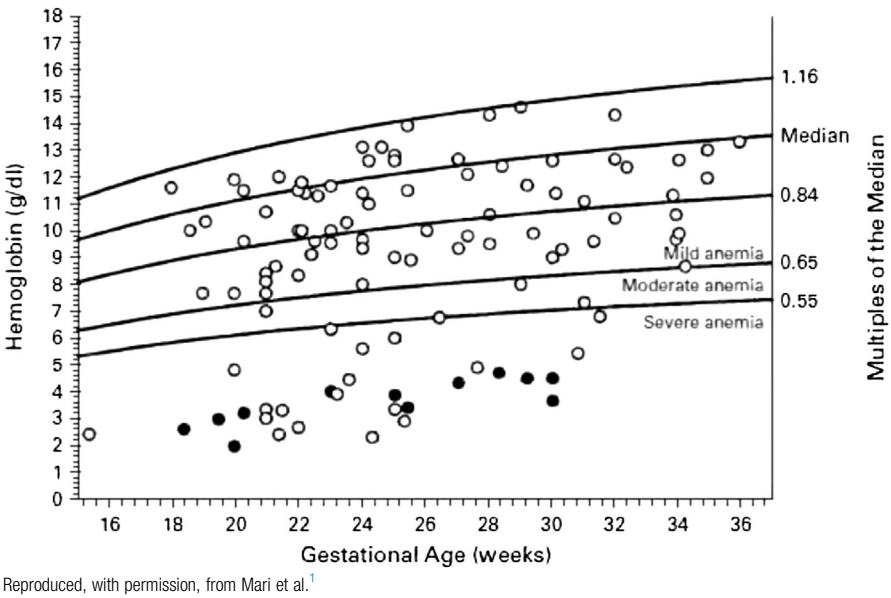
What is the definition of fetal anemia?

Fetal anemia can be defined using either hemoglobin or hematocrit values. A hemoglobin value that is more than 2 SD below the mean is diagnostic of fetal anemia. Normally, fetal hemoglobin concentration increases with advancing gestation (Figure 1).[1] Reference ranges for fetal hemoglobin concentrations as

FIGURE 1
Relationship between fetal hemoglobin across gestational age



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a function of gestational age (from 18 to 40 weeks of gestation) have been established using fetal blood sampling (Table 1).[1]

The severity of fetal anemia can be categorized based on hemoglobin concentrations expressed as multiples of the median (MoM) for gestational age as mild (MoM 0.83−0.65), moderate (MoM 0.64−0.55), and severe (MoM <0.55).[1] Severe anemia can lead to hydrops fetalis and fetal death. Hydrops related to anemia is rare in fetuses with hemoglobin concentrations greater than 5 g/dL,[2] a value corresponding to 0.47 MoM at 18 weeks of gestation and 0.36 MoM at 37 weeks of gestation. Using a fetal hematocrit of less than 30% as a cutoff for fetal anemia appears equally reliable as using hemoglobin levels and is often used in routine clinical care.[3]

What are the causes of fetal anemia?

Fetal anemia can result from a large number of pathologic processes (Table 2). The most common causes in the United States are maternal alloimmunization and parvovirus infection. Other causes include inherited conditions such as alpha-thalassemia and genetic metabolic disorders as well as acquired conditions, such as fetal blood loss and infection. Fetal anemia can occur in association with Down syndrome, because of transient abnormal myelopoeisis, a leukemic condition that occurs in approximately 10% of infants with Down syndrome.[4,5] Vascular tumors and arteriovenous malformations of the fetus or placenta are also rare causes of fetal anemia.[6,7]

Maternal red blood cell alloimmunization occurs when the immune system is sensitized to foreign erythrocyte surface antigens, stimulating the production of immunoglobulin G (IgG) antibodies. These IgG antibodies can cross the placenta and lead to hemolysis if the fetus is positive for the specific erythrocyte surface antigens. This process, known as hemolytic disease of the fetus and newborn, can result in extramedullary hematopoiesis, reticuloendothelial clearance of fetal erythrocytes, fetal anemia, hydrops fetalis, and fetal death.

The most common routes of maternal alloimmunization are blood transfusion or fetomaternal hemorrhage associated with delivery, trauma, spontaneous or induced abortion, ectopic pregnancy, or invasive obstetric procedures. The introduction of Rh (D) immune globulin in 1968 has greatly decreased the incidence of fetal anemia caused by Rh (D) alloimmunization in North America. As a result, other alloantibodies have increased in relative importance. These include antibodies to other antigens of the Rh blood group system (c, C, e, E) and other atypical antibodies also known to cause severe fetal anemia, such as anti-Kell (K, k), anti-Duffy (Fy^a), and anti-Kidd (Jk^a, Jk^b) (Table 3).

Parvovirus is the most commonly reported infectious cause of fetal anemia.[8-11] In the fetus, the virus has a predilection for erythroid progenitor cells, leading to inhibition of erythropoiesis and resultant anemia. The risk of a poor outcome for the fetus is greatest when the congenital infection occurs before 20 weeks of gestation. The risk of fetal death has been reported to be 15% at 13−20 weeks of gestation, and 6% after 20 weeks of gestation.[12] In most cases, the anemia is transient, but in severe cases, fetal intravascular transfusion may be needed to support the fetus through this aplastic crisis.

A number of viral, bacterial, and parasitic infectious diseases, including toxoplasmosis, cytomegalovirus (CMV), coxsackie virus, and syphilis, have in rare cases been associated with fetal anemia and hydrops.[13,14]

Fetal anemia can occur as a complication of monochorionic twin pregnancies, a condition referred to as twin anemia-polycythemia sequence.[15,16] This condition has been reported to occur spontaneously in 3−5% of monochorionic twins or after laser therapy for twin-twin transfusion syndrome (TTTS) in 13% of cases.[17] Twin anemia-polycythemia sequence is distinct from TTTS because it occurs in the absence of amniotic fluid abnormalities characteristic of classical TTTS. Fetal anemia can also result from fetomaternal hemorrhage, which may occur as an isolated acute event or as a chronic, ongoing hemorrhage.[18-21]

Several inherited disorders are associated with fetal anemia.[22,23] Alpha-thalassemia is the most common of these and occurs primarily in individuals of Southeast Asian descent. The severe hemolytic anemia associated with

alpha-thalassemia typically leads to hydrops fetalis and fetal demise. Less common causes of fetal anemia and hydrops include erythrocyte enzymopathies such as glucose-6-phosphate dehydrogenase deficiency, pyruvate kinase deficiency, and maternal acquired red cell aplasia.[24,25] Genetic conditions associated with aplastic anemia that may present in fetal life include Fanconi anemia and Diamond Blackfan anemia.[26,27] Inherited metabolic disorders, particularly lysosomal storage diseases such as various mucopolysaccaridoses, Gaucher disease, and Niemann-Pick disease have also been reported to cause fetal anemia and hydrops.[28]

What is the appropriate management for the patient at risk for fetal anemia?

Women with pregnancies with the conditions listed in Table 2, most commonly red blood cell alloimmunization and parvovirus infection, are considered at risk for fetal anemia. The management of such patients is based on the suspected etiology. In women with red cell alloimmunization, parental assessment and testing are key initial steps to determine the potential fetal antigen status (Figure 2). This can be done through parental zygosity testing, direct genotyping of the fetus with amniocentesis, or noninvasive fetal genotyping from maternal blood using cell-free DNA.

At this point in time, only cell-free DNA testing for Rh (D) is clinically available in the United States, whereas in Europe assays have been developed for c, E, and Kell antigens. Currently cell-free DNA testing is reported to detect the Rh (D) genotype with a sensitivity of 97.2% and a specificity of 96.8%.[29-31] Another recent study reported the accuracy for Rh (D) by trimester: 99.1% in the first trimester, 99.1% in the second trimester, and 98.1% in the third trimester.[32]

In alloimmunized women who do not undergo fetal or paternal testing and do not have a prior history of an affected pregnancy, serial antigen titers can be measured and followed up until they surpass a critical titer that places the fetus at risk for the development of severe anemia and hydrops.[3] The critical titer is set by each laboratory and may be different for various red cell antigens. Titers should be repeated serially every 4 weeks and then more frequently if they are found to be rising or with advancing gestational age. Once the critical titer is reached, 2 options exist for subsequent evaluation: fetal antigen testing (cell-free fetal DNA testing for Rh [D] or amniocentesis for fetal Rh genotyping) or initiation of ultrasound surveillance with middle cerebral artery (MCA) Doppler assessment.

The potential benefit of fetal antigen testing first is to avoid multiple serial MCA Doppler assessments (often weekly) in an antigen-negative fetus. However, cell-free DNA testing for fetal Rh (D) type is not 100% sensitive, particularly at earlier gestational ages, so a small number of at risk fetuses may be missed if this approach is chosen.[33] Although uncommon, maternal titers can increase, even in antigen-negative fetuses. Given the approximately 10% false-positive rate of MCA Doppler for the detection of severe anemia, without confirmation of fetal antigen status, women are at risk for unnecessary procedures including invasive testing. Clinicians managing alloimmunized

TABLE 1
Reference ranges for fetal hemoglobin concentrations (grams per deciliter) as a function of gestational age

Gestational age, wks	1.0 MoM, median	0.55 MoM	0.65 MoM	0.84 MoM
18	10.6	5.8	6.9	8.9
19	10.9	6.0	7.1	9.1
20	11.1	6.1	7.2	9.3
21	11.4	6.2	7.4	9.5
22	11.6	6.4	7.5	9.7
23	11.8	6.5	7.6	9.9
24	12.0	6.6	7.8	10.0
25	12.1	6.7	7.9	10.2
26	12.3	6.8	8.0	10.3
27	12.4	6.8	8.1	10.4
28	12.6	6.9	8.2	10.6
29	12.7	7.0	8.3	10.7
30	12.8	7.1	8.3	10.8
31	13.0	7.1	8.4	10.9
32	13.1	7.2	8.5	11.0
33	13.2	7.2	8.6	11.1
34	13.3	7.3	8.6	11.1
35	13.4	7.4	8.7	11.2
36	13.5	7.4	8.7	11.3
37	13.5	7.5	8.8	11.4
38	13.6	7.5	8.9	11.4
39	13.7	7.5	8.9	11.5
40	13.8	7.6	9.0	11.6

Normal hemoglobin values were 0.84 MoM or greater; mild anemia: Hgb values were between 0.65 and 0.84 MoM; moderate anemia: Hgb values were between 0.55 and 0.64 MoM; and severe anemia: Hgb values were 0.55 MoM or less.

Hgb, hemoglobin; *MoM*, multiples of the median.
Adapted from Mari et al.[1]

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TABLE 2
Potential causes of fetal anemia

Categories	Cause
Immune	Red blood cell alloimmunization Rh Atypical antigens
Infectious	Parvovirus CMV Toxoplasmosis Syphilis
Inherited	Lysosomal storage diseases (eg, mucopolysaccharidosis type VII, Niemann-Pick disease, Gaucher disease) Blackfan-Diamond anemia Fanconi anemia Alpha-thalassemia[a] Pyruvate kinase deficiency G-6-PD deficiency
Other	Aneuploidy TTTS; twin anemia-polycythemia sequence Fetomaternal hemorrhage Maternal acquired red cell aplasia

CMV, cytomegalovirus; *G-6-PD*, glucose-6-phosphate dehydrogenase; *TTTS*, twin-to-twin transfusion syndrome.

[a] Alpha-thalassemia is a common cause of hydrops in regions where this inherited disorder is common, such as Southeast Asia.

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women should be aware of these potential issues.

In women who are at risk for fetal anemia caused by parvovirus exposure, maternal antibody status (eg, immunoglobulin M positive status or IgG seroconversion) is useful to determine prior exposure and the presence of immunity. Although the peak risk for hydrops is 4–6 weeks after maternal infection, weekly evaluation of MCA Doppler studies and ultrasound surveillance for fetal hydrops are often continued for up to 10–12 weeks after exposure.

How is the diagnosis of fetal anemia made?

An algorithm for the screening and diagnosis of fetal anemia is presented in Figure 2. The definitive diagnosis of fetal anemia is generally made by fetal blood sampling, whereas screening is performed with MCA Doppler.

Diagnostic methods

Fetal anemia can be directly diagnosed by fetal blood sampling in fetuses with hydrops or in cases that have surpassed the critical threshold for MCA Doppler values (Table 4) and are thereby at significant risk.[34,35] These procedures carry potential risk to the fetus and mother of infection, preterm premature rupture of membranes, abruption, premature labor, fetal or maternal bleeding, worsening alloimmunization, and fetal death. Although the risk of fetal loss because of fetal blood sampling is reported to be 1–2%, it is gestational age dependent, with earlier gestations at higher risk.[36]

The use of delta optical density 450 to detect fetal anemia is primarily of historic interest.[37,38] In the past, the diagnosis of fetal anemia in cases of red cell alloimmunization associated with hemolysis was based on spectrophotometric measurement of the amniotic fluid for increased bilirubin concentration.[39,40] In rare cases in which MCA Doppler studies cannot be performed, measuring the delta optical density 450 levels in amniotic fluid as a screening test for fetal anemia may be reasonable, although the accuracy is limited in some circumstances, such as with anti-Kell alloimmunization. An algorithm for using delta optical density 450 is available in the medical literature.[41]

Screening methods

Independent of etiology, fetal anemia can be detected by Doppler ultrasonography on the basis of an increase in the peak velocity of systolic blood flow (PSV) in the MCA.[42] Although there is not a strong correlation between MCA–peak systolic velocity (MCA-PSV) and fetal hemoglobin concentration when the fetus is not anemic or is only mildly anemic, as the hemoglobin decreases, the MCA-PSV increases and can be used to determine the hemoglobin value with a good level of approximation.[43,44]

A MCA-PSV of greater than 1.5 MoM is used as a screening test to identify the severely anemic fetus. In one of the first large multicenter studies, including 111 fetuses at risk for anemia and 265 nonanemic fetuses, Mari et al[1] reported a sensitivity of a single value of MCA-PSV of nearly 100% (95% confidence interval, 0.86–1.0) for moderate or severe anemia with a false-positive rate of 12%.

In 2009, Pretlove et al[45] published a metaanalysis on the diagnostic value of MCA Doppler flow studies for fetal anemia. Twenty-five studies with 1639 participants were included. Of 9 studies from which the data could be pooled, a sensitivity of 75.5% and a specificity of 90.8% were reported for detecting severe anemia. The use of the MCA-PSV trends (as opposed to a single measurement) may decrease the false-positive rate to less than 5%.[44]

Although the MCA-PSV was initially developed to screen for fetal anemia caused by red cell alloimmunization, it has been demonstrated to be useful in the assessment of fetal anemia from other causes, such as parvovirus, twin-twin transfusion syndrome, and fetomaternal hemorrhage.[45-50]

What are optimal techniques for performing a measurement of the MCA-PSV?

Operators should be trained to measure the MCA-PSV using the proper technique.[51] A step-by-step video tutorial is available at SMFM.org/AJOG.org (Video). The steps for correct measurement of the MCA-PSV are the following:

1. Obtain an axial section of the fetal head at the level of the sphenoid bones during a period of fetal rest.
2. Image the circle of Willis with color Doppler.
3. Select the area of the MCA close to the transducer.
4. The entire length of the MCA should be visualized.
5. Zoom the area of the MCA-PSV in such a way that the MCA occupies more than 50% of the image.
6. The MCA-PSV should be sampled close to its origin from the internal carotid artery.
7. Ideally, the angle between the direction of blood flow and the ultrasound beam should be as close to zero as possible and parallel to the artery for the entire length, without the need for angle correction.
8. The MCA flow velocity waveforms are displayed and the highest point of the waveform (PSV) is measured.

This sequence should be repeated at least 3 times in each fetus and the highest MCA-PSV used for clinical care. The time required for the procedure is approximately 5–10 minutes. The fetus should be in a quiescent state (no breathing or movements) because of the potential effects of changes of the fetal heart rate that may have an impact on the MCA-PSV.[52] Whereas for the insonation of the MCA-PSV, the optimal angle is 0 degrees, this is not always possible because of fetal positioning and movement. In this situation, angle correction may be a reasonable approach.[53]

How often the MCA-PSV should be repeated depends on prior history, gestational age, and measured MCA-PSV MoM level. Surveillance should be reserved for a time that the pregnancy is advanced enough such that a fetal blood sampling procedure or intrauterine transfusion can technically be completed, typically 18–20 weeks of gestation. After 24 weeks of gestation, routine testing is usually done on a weekly basis but may be done more frequently with higher MoM levels or other abnormal ultrasound findings that are suggestive of developing anemia.

TABLE 3
Non–Rh (D) antibodies and associated hemolytic disease newborn and fetus

Antigen system	Specific antigen	Antigen system	Specific antigen	Antigen system	Specific antigen
Frequently associated with severe disease					
Kell	-K (K1)				
Rhesus-c					
Infrequently associated with severe disease					
Colton	-Coa	MNS	-Mta	Rhesus	-HOFM
	-Co3		-MUT		-LOCR
Diego	-ELO		-Mur		-Riv
	-Dia		-Mv		-Rh29
	-Dib		-s		-Rh32
	-Wra		-sD		-Rh42
	-Wrb		-S		-Rh46
Duffy	-Fya		-U		-STEM
Kell	-Jsa		-Vw		-Tar
	-Jsb	Rhesus	-Bea	Other antigens	-HJK
	-k (K2)		-C		-JFV
	-Kpa		-Ce		-JONES
	-Kpb		-Cw		-Kg
	-K11		-Cx		-MAM
	-K22		-ce		-REIT
	-Ku		-Dw		-Rd
	-Ula		-E		
Kidd	-Jka		-Ew		
MNS	-Ena		-Evans		
	-Far		-e		
	-Hil		-G		
	-Hut		-Goa7		
	-M		-Hr		
	-Mia		-Hro		
	-Mit		-JAL		
Associated with mild disease					
Dombrock	-Doa	Gerbich	-Ge2	Scianna	-Sc2
	-Gya		-Ge3	Other	-Vel
	-Hy		-Ge4		-Lan
	-Joa		-Lsa		-Ata
Duffy	-Fyb	Kidd	-Jkb		-Jra
	-Fy3		-Jk3		

Reproduced, with permission, from Moise.[69]

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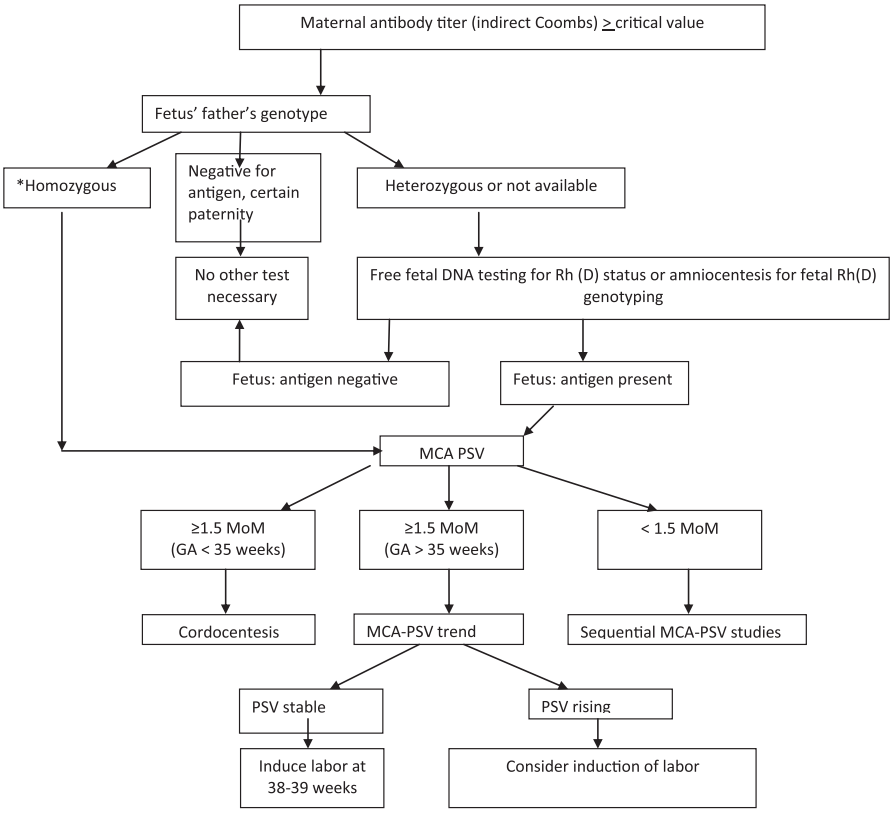
FIGURE 2
Algorithm for clinical management of the red cell alloimmunized pregnancy



GA, gestational age; *MCA*, middle cerebral artery; *MoM*, multiples of the median; *PSV*, peak systolic velocity.
Modified from Moise and Argoti.[77]
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TABLE 4
Expected peak velocity of systolic blood flow in the middle cerebral artery as a function of GA

	Multiples of the median, cm/s			
GA, wks	1.0	1.29	1.50	1.55
18	23.2	29.9	34.8	36.0
20	25.5	32.8	38.2	39.5
22	27.9	36.0	41.9	43.3
24	30.7	39.5	46.0	47.5
26	33.6	43.3	50.4	52.1
28	36.9	46.6	55.4	57.2
30	40.5	52.2	60.7	62.8
32	44.4	57.3	66.6	68.9
34	48.7	62.9	73.1	75.6
36	53.3	69.0	80.2	82.9
38	58.7	75.7	88.0	91.0
40	64.4	83.0	96.6	99.8

GA, gestational age; *MoM*, multiples of the median.
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After anemia is detected, what is the management?

An algorithm for the diagnosis of fetal anemia in the pregnancy complicated by red cell alloimmunization is depicted in Figure 2.

If the fetus is deemed at a significant risk for severe anemia based on the MCA Doppler, fetal blood sampling should be offered after counseling the parents.[35] It is important to have a coordinated team effort among individuals familiar with fetal blood sampling and intrauterine fetal transfusion. Referral to a center with expertise in invasive fetal therapy is recommended.

Determining the quantity of blood to transfuse is calculated using one of many standard formulas or charts. In fetuses over 24 weeks of gestation, a simple method for calculating the volume of donor blood to be transfused uses a coefficient multiplied by the estimated fetal weight in grams to increase the fetal hematocrit by specific increments (Table 5).[54] For example, with an initial hematocrit of 20%, the coefficient for raising the hematocrit to 40% is 0.04. In a 1000 g fetus, therefore, multiplying 1000 times 0.04 equals a transfusion volume of 40 mL. This formula assumes an approximate hematocrit of the donor blood of 75%.

Estimated fetal weight (EFW)(grams)
× coefficient (table)
= volume to transfuse

Giannina et al[54] compared several methods of calculating transfusion volume, and the choice of which to use is a matter of ease and familiarity by the operator performing the transfusion (Tables 5-7). Formulas to determine the optimal transfusion volume typically consider the hematocrit of the donor unit, the estimated weight of the fetus, and the target final fetal hematocrit,

TABLE 5
Method for calculating volume for fetal transfusion using transfusion coefficient

Desired increment in hematocrit, %	Transfusion coefficient
10	0.02
15	0.03
20	0.04
25	0.05
30	0.06

EFW (grams) × coefficient (table) = volume to transfuse

EFW, estimated fetal weight.
Adapted from Moise et al.[78]
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which should all be determined before the procedure. The fetal hematocrit at sampling, the fetal condition (eg, presence of hydrops), and the gestational age also affect the optimal volume of blood to be given. In general, the final target hematocrit should be approximately 40–50%.

Transfusion to higher levels may allow a longer interval between transfusions, but higher blood viscosity at supraphysiological hematocrit levels has been associated with a theoretical increased risk of complications.[55,56] If the fetal hematocrit is greater than 30%, no transfusion is necessary, although a resampling may be recommended within 1–2 weeks, depending on the clinical circumstances.

Blood for intrauterine transfusions undergoes the same testing that occurs for any red cell donor unit, in addition to specific testing and preparation. For intrauterine transfusions, type O Rh (D) negative blood is most often transfused; blood negative to other antigens, such as Kell, may be necessary at times. The units are screened to assure they are CMV negative, and relatively fresh units are used to assure the optimal levels of 2,3-dephosphoglycerate. Blood is irradiated to minimize the risk of graft vs host reaction, and leukodepletion is also used. Tightly packed donor cells (hematocrit of 75–85%) are typically used to minimize the total required volume.

Severely anemic fetuses at 18–24 weeks of gestation are at high risk of complications from intrauterine transfusion. It has therefore been suggested that the posttransfusion hematocrit at these early gestations should not exceed 25% or a 4-fold increase from the pretransfusion value.[57] If needed, a second intrauterine transfusion can be performed within 48 hours to bring the fetal hematocrit into the normal range, and a third procedure scheduled in 7–10 days. It has also been suggested that in fetuses at less than 22 weeks' gestation, an intraperitoneal transfusion may be a safer approach.[58]

The formula for determining the volume of intraperitoneal transfusion considers the maximum infusion of red cells that will not result in excessive intraabdominal pressure and therefore compromise umbilical venous blood flow. A simple formula subtracts 20 from the gestational age in weeks and multiplies by 10.[59] As an example, a 30 week fetus would receive 100 mL of blood ([30 weeks − 20] × 10 = 100 mL). Blood in the peritoneal cavity is absorbed over a 7–10 day period.

There are various set-ups and variations in technique for performing a fetal blood sampling and transfusion. Our prior SMFM Clinical Guideline on fetal blood sampling provides further details.[35] Preprocedure considerations include the administration of corticosteroids for fetal lung maturity in a viable fetus at less than 34 weeks of gestation in which delivery would be undertaken if necessary. Furthermore, if the pregnancy is viable, the procedure should be performed in a suite in close proximity to the operating room, alerting staff that an emergent cesarean birth may be needed, or in the operating room itself if the ultrasound unit is remote from the delivery room. Maternal intravenous access is necessary if a cesarean birth could be performed for fetal bradycardia or if intravenous sedation is used.

Although some practitioners choose to use antibiotic prophylaxis when performing an intrauterine transfusion, there are no studies evaluating the efficacy of antibiotics for this procedure.

TABLE 6
Predicted volume of PRBC required for desired level of hematocrit increase according to EFW

	Level of desired increase in hematocrit				
EFW, g	10%	15%	20%	25%	30%
500	12.5	16.1	19.7	23.2	26.8
600	14.8	19.1	23.4	27.7	32.0
700	17.2	22.2	27.2	32.2	37.2
800	19.5	25.2	31.0	36.7	42.4
900	21.8	28.3	34.7	41.2	47.6
1000	24.2	31.3	38.5	45.7	52.8
1100	26.5	34.4	42.3	50.1	58.0
1200	28.8	37.4	46.0	54.6	63.2
1300	31.2	40.5	49.8	59.1	68.4
1400	33.5	43.5	53.5	63.6	73.6
1500	35.8	46.6	57.3	68.1	78.8
1600	38.1	49.6	61.1	72.5	84.0
1700	40.5	52.7	64.8	77.0	89.2
1800	42.8	55.7	68.6	81.5	94.4
1900	45.1	58.7	72.4	86.0	99.6
2000	47.5	61.8	76.1	90.5	104.8
2100	49.8	64.8	79.9	94.9	110.0
2200	52.1	67.9	83.7	99.4	115.2
2300	54.5	70.9	87.4	103.9	120.4
2400	56.7	73.9	91.0	108.2	125.4
2500	59.0	76.9	94.8	112.7	129.6

EFW, estimated fetal weight; *PRBC*, packed red blood cells.
Reproduced, with permission, from Plecas et al.[70]

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TABLE 7
Predicted volume of PRBC required for desired level of hematocrit increase according to GA

	Level of desired increase in hematocrit				
GA, wks	10%	15%	20%	25%	30%
21	13.1	14.2	15.2	16.3	17.3
22	13.7	15.8	17.9	19.9	22.0
23	14.8	17.9	21.1	24.2	27.3
24	16.5	20.6	24.8	30.0	33.1
25	18.7	23.9	29.1	34.3	39.5
26	21.4	27.7	33.9	40.2	46.4
27	24.7	32.0	39.3	46.6	53.9
28	28.6	36.9	45.3	53.6	61.9
29	33.0	42.4	51.7	61.1	70.5
30	37.9	48.4	58.8	69.2	79.6
31	43.4	54.9	66.4	77.8	89.3
32	49.5	62.0	74.5	87.0	99.5
33	56.0	69.6	83.2	96.7	110.3
34	63.2	77.8	92.4	107.0	121.6

GA, gestational age; *PRBC*, packed red blood cells.
Reproduced, with permission, from Plecas et al.[70]

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An example of a typical set-up for an intrauterine transfusion as well as a step-by-step guide to performing the procedure is presented in Tables 8 and 9.

Once an intrauterine transfusion has been performed, a second transfusion is often necessary, especially if the fetus is remote from term. The need for serial or subsequent transfusions is typically less in the setting of parvovirus infection compared with alloimmunization. After a transfusion in an alloimmunized pregnancy, the fetal hemoglobin will drop at approximately 0.4 g/dL per day and the hematocrit at approximately 1% per day.

The timing of a second transfusion can be difficult to determine with certainty, but it appears that using the MCA-PSV can give an accurate assessment of when to resample the fetus. Detti et al[60] reported that the MCA-PSV was able to detect severe anemia with 100% sensitivity and a false-positive rate of 6% and thus accurately predict the need for and timing of the second transfusion.

Following an initial transfusion, the recommended threshold for the diagnosis of fetal anemia requiring a second transfusion is higher (MoM >1.69), most likely because of the contribution of donor blood given as part of the initial intrauterine transfusion. As an alternative, if the posttransfusion hematocrit is known or can be estimated, the timing of the next transfusion can be calculated using the expected decline in fetal hematocrit. Subsequent to a second transfusion, the intertransfusion interval should be individualized based on the underlying pathology, fetal condition, and posttransfusion fetal hematocrit rather than MCA-PSV thresholds.

What is the appropriate timing of delivery for the fetus at risk for anemia?

Unfortunately, there are no high-quality data regarding the optimal timing of delivery in the fetus at risk for anemia or in the fetus receiving in utero therapy because of anemia. Expert opinion suggests planning delivery at 37–38 weeks of gestation based on balancing the risk of stillbirth, the consequences of fetal anemia, and the risks of another fetal blood sampling procedure/intrauterine transfusion, against the risks of prematurity and the additional morbidity of anemia and hyperbilirubinemia prior to term delivery. Therefore, most clinicians will perform the last fetal blood sampling and transfusion no later than 34–35 weeks of gestation.

The goal is to deliver a fetus with no or only mild to moderate anemia. However, proper preparation for delivery is as important as the antenatal approach. Although neonatal services should be involved early in the care of these patients, it is particularly critical for them to be prepared for delivery so that blood for transfusion can be ready if needed and the proper personnel can be present at delivery.

What are the short and long outcomes for neonates following treatment of fetal anemia?

Short-term neonatal outcomes after treatment

With the use of intrauterine transfusions (IUTs), overall perinatal mortality in severe fetal anemia has decreased to less than 10%. Postnatal management of hemolytic disease of the newborn is primarily centered on the treatment of hyperbilirubinemia with phototherapy and exchange transfusions to prevent kernicterus. Other short-term complications include neonatal anemia, thrombocytopenia, cholestasis, and respiratory disease. Neonates who have undergone multiple intrauterine transfusions are generally born with an absence of reticulocytes because of a red cell population consisting mainly of transfused red cells containing adult hemoglobin. Therefore, these neonates may become anemic and require top-off transfusions in the first few weeks after birth.

In a review of all cases of intravascular transfusion for red cell alloimmunization over 20 years in Stockholm, Sweden, the authors reported on the outcomes of 284 in utero transfusions in 86 pregnancies in 72 women. There were 80 live births with a median gestational age

TABLE 8
Example of a fetal blood sampling and transfusion set-up

1. Obtain O negative, CMV-negative, irradiated packed red blood cells from the blood bank. O positive blood may be needed when antibodies to the c antigen are present because the rate of O negative and c negative blood is very rare (0.0001%).
2. Under sterile conditions open:
   a. Four paper or cloth drapes or single sterile drape (as used for cesarean delivery).
   b. Towel clips as needed.
   c. Twenty- or 22-gauge spinal needle (22 gauge for transfusions <24 weeks of gestation or if thrombocytopenia is suspected) prepared with heparin to prevent clot formation.
      i. Length of needle is determined ahead by measuring distance on ultrasound from maternal abdominal wall to cord insertion site.
   d. Sterile ultrasound probe cover.
   e. Sterile ultrasound gel.
   f. A skin preparation solution (betadine- or chlorhexidine-based solution).
   g. Eight to 10 1 mL syringes flushed with heparin to avoid clot formation.
   h. One 1 mL syringe for paralytic agent (atracurium or vecuronium).
   i. Five to 10 20 mL syringes (for storing blood).
   j. Four 12 mL syringes.
   k. One 3 mL syringe.
   l. Three needles 18 or 20 gauge for drawing blood from blood bank into 20 mL syringes.
   m. One 22- or 25-gauge needle.
   n. A 5.5 inch small bore extension set with t-connector and luer adaptor).
   o. Three-way stopcock.
3. Fill two 5 mL syringes with physiological saline solution.
4. Flush 1 mL syringes with heparin, save one unflushed 1 mL syringe for vecuronium (or atracurium).
5. Draw up normal saline to make 3 saline flushes, remove air bubbles by holding syringes upright and tapping to release bubbles to top, attach small bore connection tubing, and flush air through.
6. Reconstitute vecuronium with 10 mL of normal saline.
   a. Draw up 1 mL of vecuronium and 9 mL of normal saline in a 12 mL syringe.
   b. Transfer 1 mL of vecuronium mixture to a unheparinized 1 mL syringe.
   c. Mark both the 12 mL and 1 mL syringes with vecuronium to avoid confusion.
   d. Usual dose of vecuronium is 0.1 mg/kg and atracurium is 0.4 mg/kg.
7. Draw up 2% lidocaine in 3 mL syringe, attached to 22- or 25-gauge needle for injection at puncture site for maternal local anesthesia.
8. Care should be taken to maintain sterility when drawing up solutions: either have an assistant holding saline, vecuronium, lidocaine, and blood from blood bank or use single operator technique keeping one hand sterile and one hand unsterile.
9. Attach intravenous connection tubing to unit of packed red blood cells.
10. Attach stopcock, taking care to maintain sterility on one end of the stopcock.
11. Fill 20 mL syringes with blood by opening stopcock.
    a. Remove any air bubbles that may be present by holding syringes upright and tapping side of syringe to release air bubbles.
12. Have tubes available to send for laboratory studies.
    a. Remember to include not only initial, midway, and final blood counts plus any additional tubes for genetic studies, liver function studies, or other tests.

CMV, cytomegalovirus.

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at delivery of 36 weeks of gestation (range, 28–40 weeks of gestation), and 19 (23.8%) infants were born prior to 34 weeks of gestation. The median duration of neonatal hospitalization was 8 days (range, 0–64), whereas 61.2% of neonates were treated with exchange transfusions and 97.5% required phototherapy. During the neonatal intensive care unit stay, 28.8% received top-up transfusions.

Newborns are also at risk for neonatal cholestasis with elevated levels of conjugated bilirubin. The pathogenesis of cholestasis in hemolytic disease of the newborn is not well understood but may be related to the multiple in utero and postnatal transfusions leading to hyperferritenemia and iron overload in the liver.

Long-term outcomes

Maternal

The mainstay of treatment for severe fetal anemia is intrauterine blood transfusion. Unfortunately, this therapy is associated with a risk of immunization to additional antigens, despite the relatively small amount of blood transfused. In one large cohort, 25% of women formed additional antibodies after IUT, and more than 70% had multiple red blood cell antibodies postpartum.[61] The risk is highest when the IUT requires transplacental passage of the needle. The presence of multiple antibodies in the mother may make exact blood type cross-matching difficult, and this can be problematic if she requires transfusions at delivery or later.

Fetus/neonate

Recent advances in the treatment of fetal anemia caused by alloimmunization have reported survival rates close to 90%, although this percentage varies with the experience of the operator, the particular center, and

TABLE 9
Example of step-by-step guide for performing fetal blood transfusion

1. Perform ultrasound to select site.
   a. Placental cord insertion, free loop, umbilical cord insertion or intrahepatic vein.
   b. Obtain measurement from maternal abdomen to umbilical vein site of puncture to ensure correct needle length.
   c. Document fetal heart rate.
2. Have sonographer and assistant ready in addition to main operator.
3. Obtain maternal sample of blood.
4. Intravenous access and use of antibiotics is not always necessary and is at the preference of the operator.
5. Wash hands.
6. Under aseptic conditions prepare patient with antibacterial solute and place drapes leaving abdomen exposed.
7. Cover ultrasound transducer with sterile cover.
8. Identify site of puncture.
9. Give local anesthesia to patient (mother).
10. Inject fetus with intramuscular paralytic agent if necessary (vecuronium or atracurium).
11. Use 20- or 22-gauge needle to enter umbilical vein.
12. Remove stylet.
13. If flow is immediate, obtain sample in 1 mL syringe and send to laboratory.
14. If flow is not immediate and you think you are in Wharton's jelly, slowly and carefully reposition the needle to enter into the vein.
15. Some operators document flow by injecting saline: if that is done prior to obtain fetal blood sample, discard first 1 mL fetal blood because it may be diluted with saline.
16. Document fetal blood sample by comparing maternal (previously drawn and analyzed) and fetal hematocrit and MCV.
    a. This may not be necessary if sampling a free loop or the intrahepatic vein or if document flow with saline.
17. Attach tubing to transfuse slowly: assistant can push blood slowly; watch segment of umbilical cord to see if blood is flowing through umbilical vein.
    a. A small slow transfusion of blood may be performed prior to obtaining confirmatory results of fetal blood from the laboratory to prevent clot from forming.
18. When the fetal hematocrit returns and a transfusion is needed, calculate the amount of blood needed to transfuse.
19. Intermittently obtain fetal heart rate.
20. If transfusing a large amount of blood, consider getting a midprocedure hematocrit.
21. When transfusion is complete, obtain final hematocrit, and draw any other blood needed for work-up. Some practitioners will perform a Kleihauer Betke test for percentage of fetal and adult red blood cells in the final hematocrit.
22. Some operators choose to also perform an intraperitoneal transfusion, which allows slow absorption of blood over 7–10 days and may prolong time until next transfusion.
    a. If performing intraperitoneal transfusion, calculate amount of blood needed by the following formula: GA (weeks) $-$ 20 $\times$ 10. For example, at 30 weeks, 30 $-$ 20 $=$ 10 $\times$ 10 $=$ 100 mL blood.
23. After the transfusion is complete and the needle is removed, watch the puncture site for streaming and check fetal heart rate for bradycardia.
24. Consider monitoring the patient, and fetus if indicated, after transfusion for 1 hour.

GA, gestational age; *MCV*, mean corpuscular volume.

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the presence of hydrops. Some of these advances have led to the survival of severely anemic and hydropic fetuses, with the concern that improved survival may lead to an increase in long-term morbidity.

Several small studies have reported on the long-term outcomes of infants born after IUT. These studies have

TABLE 10
Long-term follow-up of newborn outcomes following IUT

Author (year)	n	Follow-up duration	Cerebral palsy	Hearing loss	Severe developmental delay	Mild developmental delay
Doyle et al[71] (1993)	38	2 y	2.6%	7.8%	2.6%	2.6%
Janssens et al[72] (1997)	69	6 mo to 6 y	4.3%	4.3%	7.2%	8.6%
Hudon et al[73] (1998)	40	Mean 14.4 mo	2.5%	2.5%	2.5%	n/a
Grab et al[74] (1999)	30	6 y	0	0	0	3.3%
Farrant et al[75] (2001)	36	2 y	2.8%	0	0	n/a
Harper et al[76] (2006)	16	Mean 9.5 y	6.3%	6.3%	6.3%	n/a
Lindenburg et al[62] (2012)	291	Median 8.2 y	2.1%	1.0%	3.1%	11%

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varied in the length of follow-up of the children, from 6 months to 12.2 years. Three studies provided a comparison group and documented no major differences in outcomes, including sensorineural hearing loss, global developmental quotients, or other adverse serious outcomes.

The LOTUS study (LOng-Term follow-up after intra-Uterine transfusionS) is the largest study to date investigating neurodevelopmental outcomes in children treated with IUT for hemolytic disease of the fetus/newborn.[62] The cohort included 291 children aged 2−17 years who underwent IUT for red cell alloimmunization over a 20 year period between 1988 and 2008. Alloimmunization was due to Rh (D) in 80%, Kell in 12%, c in 5%, and other antibodies in 2%.

TABLE 11
Summary of outcomes in fetuses with hydrops or severe anemia and IUT caused by parvovirus

Author (year)	n	Survival	Abnormal developmental outcome
Miller et al[10] (1998)	7	42.9%	0
Dembinski et al[66] (2002)	37	83.8%	0
Nagel et al[65] (2007)	24	66.7%	31.0%
De Jong et al[64] (2012)	44	73.0%	21.4%

IUT, intrauterine transfusion.

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Overall survival was 90%; the incidence of neurodevelopmental impairment (cerebral palsy, severe developmental delay, deafness, and/or blindness) was 4.8% and was increased in the setting of fetal hydrops (mild hydrops: odds ratio [OR], 4.3; 95% confidence interval [CI], 1.2−15.3; severe hydrops: OR, 9.9; 95% CI, 2.4−40.5), and preterm birth less than 32 weeks' gestational age (OR, 12.8; 95% CI, 2.1−9.5). A summary of the long-term outcomes of newborns that had an

TABLE 12
Society for Maternal-Fetal Medicine recommendations for diagnosis and management of the fetus at risk for anemia

	Recommendations	GRADE
1	We recommend that MCA-PSV be used to as the primary technique to detect fetal anemia.	1B Strong recommendation, moderate-quality evidence
2	We recommend against the routine use of amniotic fluid delta OD450 to diagnosis fetal anemia.	1B Strong recommendation, moderate-quality evidence
3	We recommend that MCA-PSV assessment should be reserved for those patients who are at risk of having an anemic fetus.	1B Strong recommendation, moderate-quality evidence
4	Proper technique for MCA-PSV evaluation includes assessing the middle cerebral artery close to its origin, limiting overestimation of MCA-PSV, and using angle adjustment only if unavoidable.	Best practice
5	We recommend that if a fetus is deemed at significant risk for severe fetal anemia (MCA-PSV >1.5 MoM or hydropic) fetal blood sampling should be offered with preparation for IUT, unless the pregnancy is at a gestational age when risks associated with delivery are considered to be less than those associated with the procedure.	1B Strong recommendation, moderate-quality evidence
6	We recommend that if a fetus is considered at significant risk for severe fetal anemia, the patient be referred to a tertiary care center or center with expertise in invasive fetal therapy.	1C Strong recommendation, weak-quality evidence
7	We suggest that MCA-PSV be used in routine situations to determine the timing of a second transfusion in fetuses with anemia. As an alternative, if the posttransfusion hematocrit is known or can be estimated, the timing of the next transfusion can be calculated using the expected decline in fetal hematocrit. Timing of subsequent transfusions (third and beyond) should be individualized rather than based on MCA-PSV values.	2C Weak recommendation, low-quality evidence
8	We suggest that pregnancies with a fetus at risk for fetal anemia be delivered at 37−38 weeks of gestation unless indications develop prior to this time.	2C Weak recommendation, low-quality evidence

IUT, intrauterine transfusion; *MCA-PSV*, middle cerebral artery peak systolic velocity; *MoM*, multiples of the median.

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intrauterine transfusion with or without severe anemia and hydrops is presented in Tables 10 and 11.

Perinatal survival rates following IUT for parvovirus B19 seem to be lower than for alloimmunization, with studies demonstrating rates of survival ranging from 67% to 85%.[63-77] This may be due to later diagnosis of severe fetal anemia in the fetus infected with parvovirus that presents with hydrops, as opposed to the anemic fetus detected because of known maternal alloimmunization who is being followed up more closely with MCA Doppler studies.

RECOMMENDATIONS

Recommendations regarding diagnosis and management of the fetus at risk for anemia are presented in Table 12. The grading scheme classifies recommendations as either strong (grade 1) or weak (grade 2), and classifies the quality of evidence as high (grade A), moderate (grade B), or low (grade C). Thus, the recommendations can fall into 1 of the following 6 categories: 1A, 1B, 1C, 2A, 2B, 2C (Table 12).

Quality of evidence

The quality of evidence for each article was evaluated according to the method outlined by the US Preventative Services Task Force:

I	Properly powered and conducted randomized controlled trial (RCT); well-conducted systematic review or metaanalysis of homogeneous RCTs.
II-1	Well-designed controlled trial without randomization.
II-2	Well-designed cohort or case-control analytic study.
II-3	Multiple time series with or without the intervention; dramatic results from uncontrolled experiment.
III	Opinions of respected authorities, based on clinical experience; descriptive studies or case reports; reports of expert committees.

This opinion was developed by the Publications Committee of the Society for Maternal–Fetal Medicine (SMFM) with the assistance of Giancarlo Mari, MD, Mary E. Norton, MD, Joanne Stone, MD, Vincenzo Berghella, MD, Anthony Sciscione, DO, Danielle Tate, MD, Mauro H. Schenone, MD and was approved by the executive committee of the society on Nov. 19, 2014. Each member of the publications committee (Sean Blackwell, MD [Chair], Mary Norton, MD [Vice Chair], Vincenzo Berghella, MD, Joseph Biggio, MD, Aaron Caughey, MD, Suneet Chauhan, MD, Sabrina Craigo, MD, Jodi Dashe, MD, Brenna Hughes, MD, Jamie Lo, MD, Tracy Manuck, MD, Brian Mercer, MD, Eva Pressman, MD, Anthony Sciscione, DO, Neil Silverman, MD, Alan Tita, MD, and George Wendel, MD) has submitted a conflict of interest disclosure delineating personal, professional, and/or business interests that might be perceived as a real or potential conflict of interest in relation to this publication. ■

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The practice of medicine continues to evolve, and individual circumstances will vary. This opinion reflects information available at the time of its submission for publication and is neither designed nor intended to establish an exclusive standard of perinatal care. This publication is not expected to reflect the opinions of all members of the Society for Maternal—Fetal Medicine.