Original Paper

Fetal Diagnosis and Therapy

Fetal Diagn Ther
DOI: 10.1159/000501554

Received: October 25, 2018
Accepted after revision: June 18, 2019
Published online: September 10, 2019

Managing the Unusual Causes of Fetal Anemia

Emeline Maisonneuve[a, b] Imane Ben M'Barek[a] Thierry Leblanc[c] Lydie Da Costa[d] Stéphanie Friszer[a, b] Françoise Pernot[b] Pauline Thomas[a, b] Vanina Castaigne[e] Cécile Toly N'Dour[f] Agnès Mailloux[f] Anne Cortey[a, b] Jean-Marie Jouannic[a, b]

[a]Department of Fetal Medicine, Hôpital Trousseau, Paris, France; [b]Centre National de Référence en Hémobiologie Périnatale (CNRHP) Clinique, Hôpital Trousseau, Paris, France; [c]Department of Clinic Hematology, Hôpital Robert-Debré, Paris, France; [d]Department of Biologic Hematology, Hôpital Robert-Debré, Paris, France; [e]Department of Obstetrics and Gynecology, Hôpital Intercommunal de Créteil, Créteil, France; [f]Department of Immunohematology, Centre National de Référence en Hémobiologie Périnatale (CNRHP) Biologique, Hôpital Saint-Antoine, Paris, France

Keywords
Fetal anemia · Intrauterine transfusion · Fetal blood sampling · Rare cause of anemia · Hydrops fetalis

Abstract
Background: Rare causes of fetal anemia requiring intrauterine transfusion (IUT) are challenging for fetal medicine specialists. ***Objectives:*** The aim of this study was to describe the perinatal patterns and prognosis in a consecutive series of fetuses transfused for fetal anemia of rare or unknown etiology, and to propose a protocol of investigation for fetal anemia of undetermined cause and for the management of subsequent pregnancies. ***Method:*** We conducted a retrospective descriptive study on fetuses transfused for severe anemia of rare or unknown etiology managed in our national referral center (Centre National de Référence d'Hémobiologie Périnatale) and born between 2010 and 2017. ***Results:*** During the study period, 584 IUT were performed in 253 fetuses. Among those IUT, 23 (3.9%) were performed for a rare or unknown cause of anemia in 13 fetuses (5.1% of transfused fetuses). The median gestational age at diagnosis was 26 weeks of gestation (WG; range 21–33). Hemoglobin levels ranged from 1.6 to 9.1 g/dL (0.18–0.83 multiples of median) before the first IUT. The fetuses received between 1 and 6 IUT (39% received at least 2 IUT). The definitive etiologies for central anemia were: congenital syphilis, neonatal poikilocytosis, type II congenital dyserythropoietic anemia (CDA), and neonatal hemochromatosis. There was 1 case with suspected type I CDA and 1 with suspected Diamond-Blackfan anemia. There was 1 case of peripheral anemia, secondary to cerebral hemorrhages of different ages, related to a variant of the *COL4A1* gene. In 6 fetuses corresponding to 4 mothers, no precise diagnosis was found despite a complete workup. In our series, there were 8 live births, 4 terminations of pregnancy, and 1 intrauterine fetal death. ***Conclusions:*** Fetal anemia of rare or unknown diagnosis represents 5% of all transfused fetuses in our cohort. Fetal and neonatal anemias can be recurrent in further pregnancies, with variable expressivity.

KARGER

E-Mail karger@karger.com
www.karger.com/fdt

Emeline Maisonneuve, MD
Department of Fetal Medicine, Hôpital Armand Trousseau
26, avenue du Docteur Arnold Netter
FR–75012 Paris (France)
E-Mail emelinem@yahoo.com

Introduction

Fetal anemia is a severe complication of pregnancy and is mostly caused by red blood cell (RBC) alloimmunization, parvovirus B19 infection, and fetomaternal hemorrhage [1]. Since the prophylactic universal use of RhD immune globulin, anti-D alloimmunization has dramatically decreased in Western Europe and North America. Furthermore, non-invasive management of alloimmunized pregnancies, including the measurement of peak systolic velocity in the middle cerebral artery Doppler (MCA-PSV) and the fetal blood group typing from fetal DNA in maternal blood, have contributed to reduce the number of invasive procedures [2–4]. Consequently, immunological indications for intrauterine transfusion (IUT) are declining while infectious indications, as well as rare causes of fetal anemia, are increasing. Besides, in Southeast Asian populations, Bart's disease is a common cause of severe fetal anemia [5, 6]. Thus, the unusual causes of anemia requiring IUT are challenging for fetal medicine specialists and require multidisciplinary management to allow a precise diagnosis of the underlying fetal disease and appropriated parental counselling [1, 7]. In our French referral center for perinatal hemobiology, second-line investigations are systematically performed when the diagnosis of fetal anemia is uncertain at the time of the first IUT (absence of immunological or infectious context) [8]. The literature reports only 2 series of rare or unknown causes of fetal anemia, but there is no cohort with recurrent severe fetal anemia of undetermined cause [1, 9].

Our aim was to describe the perinatal patterns and prognosis in a consecutive series of fetuses with moderate to severe anemia, which required IUT, related to a rare etiology in an 8-year period. Our secondary objective was to propose a protocol of investigation for undetermined fetal anemia and the management of subsequent pregnancies.

Material and Methods

Patients

The perinatal data of 253 patients with severe fetal anemia managed in our center (CNRHP: Centre National de Référence en Hémobiologie Périnatale) between January 2010 and December 2017 were retrospectively reviewed. Moderate to severe anemia was suspected in cases with an MCA-PSV measurement over 1.5 multiple of the median (MoM) associated or not with serosal effusions [4]. Fetal anemia was confirmed at fetal blood sampling (FBS) according to the criteria defined by Mari et al. [4]. Rare or undetermined causes of fetal anemia were defined as fetal anemia not associated with maternal RBC alloimmunization, B19 parvovirus and cytomegalovirus (CMV) infections or fetomaternal hemorrhage. We also excluded severe congenital anomalies and multiple pregnancies with IUT for complications of monochorionic pregnancies.

Management of Fetal Anemia in our Center

In cases of suspected severe fetal anemia at MCA-PSV measurement, associated or not with hydrops fetalis, the treatment was emergency IUT. Before IUT, a maternal blood sample was collected to determine the maternal blood group type and for indirect anti-globulin testing, Kleihauer-Betke test, and infectious serologies (B19 parvovirus, CMV, HIV, syphilis). When this first-line analysis was negative, glucose 6 phosphate dehydrogenase (G6PD) activity (in the case of African or Mediterranean origin of the parents and grandparents) and pyruvate kinase (PK) activity were tested. Hemoglobin electrophoresis was also performed in order to identify thalassemic syndromes and hemoglobinopathies.

Ultrasonography was conducted to search for signs of fetal anemia and other congenital anomalies, including hemorrhage of a fetal organ. All the ultrasound examinations were performed by experts in fetal imaging using two types of ultrasound equipment (Voluson E8 and E10, General Electrics, Zipf, Austria; Applio, Toshiba Medical Systems, Tokyo, Japan) with transabdominal or transvaginal probes (3.5–14 MHz) when required in fetuses in cephalic presentations.

In all cases, FBS was obtained immediately prior to IUT for a per-operative fetal hemoglobin count (Hemocue®). The fetal investigation was completed on the FBS with a complete blood and reticulocyte count, to characterize the anemia and analyze abnormal RBC morphology on the blood smear [10, 11]. Aregenerative anemia was defined by an absolute reticulocyte count under 150×10^9/L [12].

FBS also included determination of fetal blood group type, as well as direct anti-globulin testing, liver function tests, parameters of hemolysis in blood (indirect bilirubin and lactate dehydrogenase), PK and G6PD activities, hemoglobin electrophoresis, erythrocyte and reticulocyte indices analyses, peripheral blood smear examination, ektacytometry, and flow cytometry for the eosin 5′ maleimide acid (EMA) test and karyotype [13]. Each test required 0.5 mL of fetal blood. The volume of fetal blood needed for a complete workup was 4 mL; in case of severe anemia, karyotype and CGH array were preferably performed on amniotic fluid, in order to spare fetal blood. FBS was also kept for further subsequent molecular screening deciphering rare to very rare causes of anemia, including Diamond-Blackfan anemia (DBA) [14, 15] or congenital dyserythropoietic anemias (CDAs) [16], or RBC membrane disorders (mostly the hereditary stomatocytosis [11]), and rare enzyme defects when the first-line analysis was negative.

Severely anemic fetuses were transfused with adult donor packed RBC, cross-matched with the mother, with a high hematocrit (70–80%) [17]. The MCA-PSV was monitored the day after the IUT and weekly until birth. A cerebral magnetic resonance imaging (MRI) was scheduled at 32 weeks of gestation (WG) in cases with Hb under 5 g/dL and at least 3 weeks after the first IUT to rule out hypoxic fetal brain damage. After birth, neonates underwent clinical and hematological follow-up by a pediatrician at the CNRHP clinics. A postmortem examination was proposed to the parents in case of termination of pregnancy (TOP) or intrauterine fetal death (IUFD).

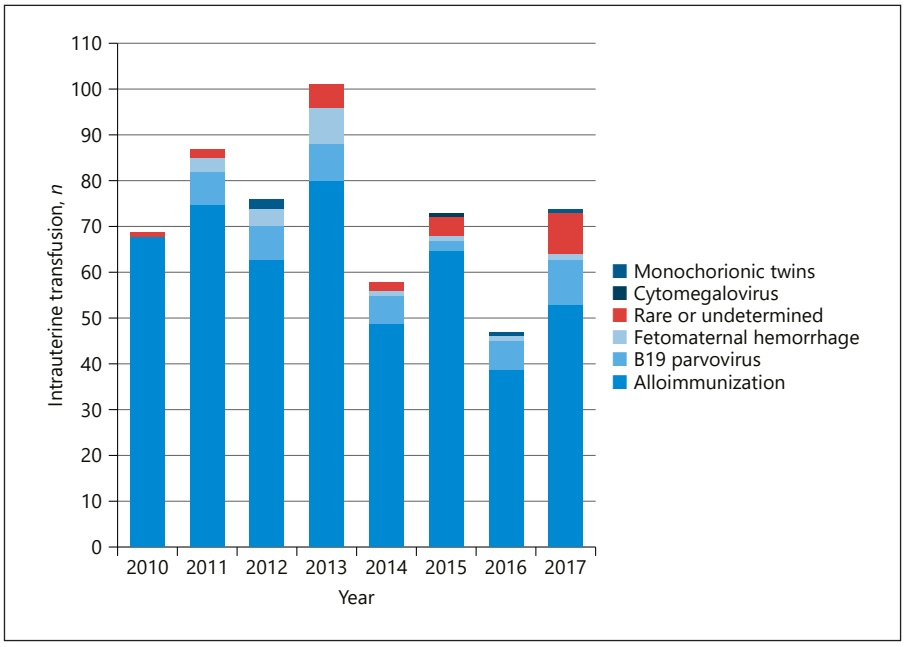

Fig. 1. Evolution of the indications for IUTs. Complications of monochorionic twin pregnancies were TAPS (twin-anemia-polycythemia-sequence).

Results

During the 8-year study period, 584 IUT were performed in 253 fetuses. Cases with indications for IUT, including RBC alloimmunization (492 IUT in 195 fetuses), B19 parvovirus infection (46 IUT in 31 fetuses), CMV infection (1 IUT in 1 fetus), fetomaternal hemorrhage (19 IUT in 9 fetuses), and complications of monochorionic twins (4 IUT in 4 fetuses) were excluded (Fig. 1). Thus, 13 fetuses presenting a rare or unknown cause of fetal anemia were included (5.1% of the transfused fetuses) requiring 23 IUT (3.9% of overall IUT).

In this cohort, no parental consanguinity was found. Details on prenatal history, FBS parameters, and outcomes are provided in Table 1. Fetal anemia was suspected during the systematic ultrasound examinations in cases 2, 4, 5, 7, 8, 9, 10, and 11. Otherwise, additional ultrasound scans were performed for specific reasons: non-reassuring FHR in case 1 within the context of reduced fetal movements; personal history of macrocytic anemia of undetermined cause in case 3; history of a previous pregnancy with neonatal anemia (case 6) or with fetal anemia (cases 8 bis and 10 bis).

The median gestational age at fetal anemia diagnosis was 26 WG (range 21–33). In 8/13 cases, anemia was suspected on a fetal hydrops and for the other 5 cases, there was elevated MCA-PSV either isolated or associated with one indirect sign of fetal anemia (hydrothorax, ascites, or cardiomegaly). The median of MCA-PSV value was 1.8 MoM at the time of the diagnosis (range 1.5–2). Hb levels ranged from 1.6 to 9.1 g/dL (corresponding to 0.18–0.83 MoM) before the first IUT. The fetuses received between 1 and 6 IUT (median 2) and the delay between each IUT varied from 3 days to 4 weeks. Only 1 IUT was necessary for 61% of fetuses, and the others received at least 2 IUT. Among the 13 cases, 9 had a cerebral MRI. It was normal for 7 fetuses and revealed brain damage in 2 fetuses for whom parents requested TOP.

Among the 13 cases, 1 IUFD occurred at 23 WG and 4 TOP were performed on parental request (indications for TOP were: [i] suspicion of CDA with hydrops and Hb = 1.6 g/dL at first IUT, [ii] severe ischemohemorrhagic cerebral lesions, [iii] hydrops associated with poor fetal movements, and [iv] cerebral white matter damage associated with hydrops, anhydramnios, and liver anomalies). There were 8 live neonates with gestational age at delivery ranging from 34 WG and 4 days to 38 WG. Two neonates were transfused 3 and 4 times after birth, respectively, but none after 6 months of postnatal age.

Second-line investigation was performed on FBS and maternal blood. When fetal anemia was aregenerative with a decreased reticulocyte count of less than 150×10^9/L, central anemia was suspected [12]. The etiologies of central anemia were: congenital syphilis (case 1) [18], neonatal poikilocytosis (pyropoikilocytosis or severe elliptocytosis, which declines with age; case 2), suspected type I CDA (CDA-I; case 3), type II CDA (CDA-II; case 4), neonatal hemochromatosis (case 5), and suspected DBA (case 6).

Table 1. Description of cases with fetal anemia of rare or undetermined cause

No.	Underlying disease	Ethnic origin, maternal and obstetric history	US findings	GA at IUT	Pre-IUT Hb, g/dL	Post-IUT Hb, g/dL	Reticulocyte count, $\times 10^9$/L	Hemolysis parameters, liver function tests, DAT	Karyo-type	G6PD PK Hb El	Ekta-cytometry, flow cytometry, peripheral blood smear	Outcome, birth weight, Hb at birth, g/dL	Postnatal evolution
1	Congenital syphilis	Sub-Saharan African, primigravida, no medical history	Strip of ascites, MCA-PSV = 1.6 MoM	34+0	8.4	16.2	30	N N negative DAT	N	N	Unknown	LB at 35 WG+2, healthy female 2,300 g Hb = 11	No postnatal transfusion, extencilline treatment
2	Poïkilocyto-sis or severe neonatal elliptocyto-sis	Sub-Saharan African, 6th gestation: 1 abortion, 2 miscarriages and 1 late loss at 18 WG in a context of hydrops	Isolated pericardial effusion at 23 WG, with evolution toward hydrops at 32 WG, MCA-PSV = 1.5 MoM	32+4	3.2	13	Not known	N N negative DAT	Not known	N	Membrane abnormal-ity	LB at 37 WG, healthy male, 3,150 g, Hb = 16.6	Not neonatal transfusion, then lost to follow-up
3	CDA-I (suspected)	Caucasian, personal history of macrocytic anemia of undetermined cause, 2nd gestation: 1 miscarriage	Cardiomegaly, MCA-PSV = 1.8 MoM, IUGR	29+4	7.8	11.1	151	N N negative DAT	N	N	N	LB at 34 WG+6 female 1,785 g (cesarean section for severe IUGR), Hb = 11.3	3 postnatal transfusions at D15, 1 month and 3 months, N develop-ment at 2 years of age
4	CDA II	Caucasian, primigravida	Hydrops, MCA-PSV = 1.5 MoM	23+0 24+0	1.6 5.2	8.6 10.8	82	Not known negative DAT	N	N	Suspicion of congenital dys-erythro-poiesis	TOP at 28 WG, male fetus 940 g	TOP
5	Neonatal hemochro-matosis	Caucasian, primigravida	Hydrothorax, MCA-PSV = 2 MoM, IUGR, white matter anomalies, small liver, anhydramnios	32+5	8.8[a]	14.6	113	N N Negative DAT	N	N	N	TOP at 34 WG	TOP
6	DBA (suspected)	North African, 5th gestation: 1 miscarriage, 1 pregnancy with neonatal anemia, 2 N pregnancies	Hydrops PSV-ACM >1.5 MoM	26+0 30+0	3.2 6.8	10.8 15.3	409	Not known negative DAT	N	N	N	LB at 38 WG male 3,000 g, Hb = 4 g/dL	4 postnatal transfusions at 0, D11, D30 and 3 months, healthy at 5 years of age
7	Variant in *COL4A1* gene	Caucasian, primigravida	Ascites, MCA-PSV = 1.6 MoM Multiple cerebral ischemohemorrhagic damage	24+4	2.3	8.1	323	Elevated bilirubin N negative DAT	N	N	N	TOP at 26 WG	TOP
8	Unknown	North African, 2nd gestation: 1 molar pregnancy	Hydrops, MCA-PSV = 2 MoM	22+2 22+4	1.8 2.6[b]	7 11.7	110	N N negative DAT	N	Minor alpha thalas-semia	N	IUFD at 23 WG	IUFD
8 bis	Unknown	Idem	Hydrops, MCA-PSV >1.5 MoM	27+6	3.3	10.4	198	Elevated bilirubin, negative DAT	N	N	N	LB at 37 WG+3, healthy male 3,560 g Hb = 18.2	No postnatal transfusion, healthy at 3 years of age
9	Unknown	Caucasian, 3rd gestation: 1 miscarriage and 1 N pregnancy	Hydrops, MCA-PSV = 1.8 MoM oligohydram-nios, decrease of fetal movements	18+5	9.1	11.1	276	Elevated bilirubin N Negative DAT	N	Minor alpha thalas-semia	N	TOP at 20 WG	TOP
10	Unknown	North African, personal history of undetermined microcytic anemia, 2nd pregnancy: history of emergency caesarian at 37 WG for fetal brady-cardia with severe neonatal anemia (Hb = 2.6 g/dL) related to severe fetomaternal hemorrhage	Nuchal translucency = 2.6 mm (>95th perc), MCA-PSV = 1.8 MoM at 32 WG	33+2	8.1	13	266	Elevated bilirubin N Negative DAT	N	N	N	LB at 37 WG+1, healthy female 3,700 g Hb = 12	No postnatal transfusion, N develop-ment at 2 years of age

Table 1 (continued)

No.	Underlying disease	Ethnic origin, maternal and obstetric history	US findings	GA at IUT	Pre-IUT Hb, g/dL	Post-IUT Hb, g/dL	Reticulocyte count, $\times 10^9$/L	Hemolysis parameters, liver function tests, DAT	Karyotype	G6PD PK Hb El	Ekta-cytometry, flow cytometry, peripheral blood smear	Outcome, birth weight, Hb at birth, g/dL	Postnatal evolution
10 bis	Unknown	Idem	Hydrops PSV-ACM = 1.9 MoM	21+3 22+22 5+0 27+4 30+2 33+1	3.6 6.9 7.3 9.4 9.5 9.5	9.2 13.4 14.9 14.1 15.9 15.2	117	N N negative DAT	N	N	N	LB at 34 WG+4, healthy male 2,300 g Hb = 11.4	No postnatal transfusion N development at 3 months of age
11	Unknown	North African, 4th gestation: 3 N pregnancies, mild alloimmunization anti-D for current pregnancy with low level of antibodies (0.4 IU/mL)	Hydrops, MCA-PSV = 1.8 MoM	28+6 29+3 31+0	3.9 7.0 9.0	8.7 13.1 15.4	244	N N negative DAT	N	N	N	LB at 37 WG, healthy female 2,990 g Hb = 14	No postnatal transfusion

CDA, congenital dyserythropoietic type anemia; D, day; DBA, Diamond-Blackfan anemia; DAT, direct antiglobulin test; El, electrophoresis; Hb, hemoglobin; G6PD, glucose-6-phosphate dehydrogenase; GA, gestational age (expressed in weeks of gestation + days); Hb, hemoglobin; IUFD, intrauterine fetal death; IUGR, intrauterine growth restriction; LB, live birth; MCA-PSV, middle cerebral artery peak systolic velocity, expressed in MoM (multiples of median); N, normal; PK, pyruvate kinase; TOP, termination of pregnancy; US, ultrasound; WG, weeks of gestation.

[a] Severe thrombocytopenia at 37×10^9/L.

[b] Pancytopenia with low leucocyte ($<1 \times 10^9$/L) and platelet ($<10 \times 10^9$/L) counts.

In case 5, fetal anemia was moderate at 8.8 g/dL at 32 WG and aregenerative (reticulocyte count = 110×10^9/L). Neonatal hemochromatosis was diagnosed on the postmortem examination: the fetus presented with hepatic fibrosis and hypoplasia with deposition of iron in the liver and pancreas.

In case 6, DBA was suspected. The mother already had 2 children born without anemia. Her third child presented a neonatal anemia (Hb = 9 g/dL at birth) and was treated with iron supplementation only. During her fourth pregnancy, two fetal transfusions were performed at 26 and 30, WG respectively. This child received 4 postnatal transfusions, at birth and at 11 days, 1 month, and 3 months of age. This child is now 5 years old and remains well and hematologically normal. The follow-up of her fifth pregnancy revealed no anomalies of the MCA-PSV measurements and this child did not present with any neonatal anemia.

In the case of fetal bleeding that was not diagnosed initially, anemia was of peripheral type. Indeed, the fetus presented moderate bilateral ventriculomegaly diagnosed before IUT for hydrops associated with elevated MCA-PSV values. Multiple cerebral hemorrhages of different ages were diagnosed at follow-up ultrasound and confirmed at postmortem examination. A de novo variant of the *COL4A1* gene was found on genetic analysis.

In the other 6 cases (corresponding to 4 different mothers), the etiologic diagnosis for fetal anemia remains unknown until now. To explore these cases of fetal anemia, complete blood cell counts (meticulous erythrocyte and reticulocyte indices analyses), reticulocyte count, Hb electrophoresis, hemolysis parameters (lactate dehydrogenase, total and indirect bilirubin, schistocyte percentage), G6PD, and PK activities were performed on parental blood. In case 3, the mother had a personal history of macrocytic anemia of undetermined cause. In case 10, the mother had a personal history of moderate transient microcytic anemia of unknown cause. In the other cases, causes of anemia, including hemolysis, were investigated, but yielded no definite diagnosis.

Discussion

During the 8-year study period, 23 IUT (3.9%) were performed for a rare or unknown cause of fetal anemia in 13 fetuses (5.1% of transfused fetuses). Among those cases the etiology of fetal anemia remains undetermined in 6/13 (46%).

To the best of our knowledge, this represents the third study focusing on rare causes of fetal anemia. First, Amann et al. [1] reported their experience on 82 transfused fetuses. Among those, no etiology was available at the time of the second IUT in 15/82 fetuses (18%). However, it should be noted that they included 4 fetuses with fetomaternal hemorrhage. Yet, we believe that the Kleihauer-Betke test or flow cytometry should be performed as a first-line examination in cases of suspected fetal anemia when alloimmunization has been ruled out. In another series, Zhang et al. [9] reported 4 cases of severe

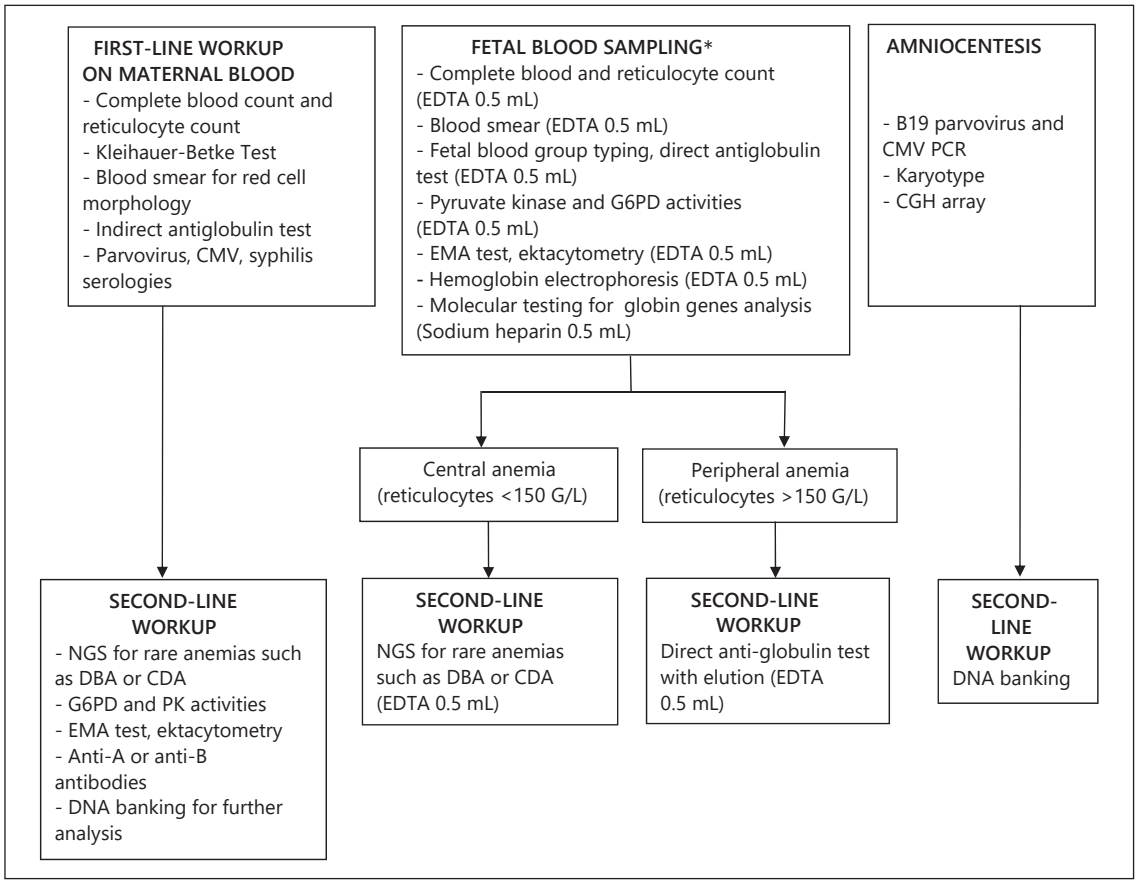

Fig. 2. Investigation of fetal anemia on maternal and fetal blood, and amniocentesis. * Before the first IUT in cases of fetal anemia of undetermined cause. CMV, cytomegalovirus; CGH array, comparative genomic hybridization; CDA, congenital dyserythropoietic type anemia; DBA, Diamond-Blackfan anemia; G6PD, glucose-6-phosphate dehydrogenase; NGS, next-generation sequencing; PK, pyruvate kinase; PCR, polymerase chain reaction; EMA test, flow-cytometry for the eosin 5′ maleimide acid test.

non-immune/infectious fetal anemia: 1 case of DBA, 1 suspicion of fetomaternal hemorrhage despite negative Kleihauer-Betke tests, and 2 cases without any diagnosis. Interestingly, we observed a much lower rate of unknown causes of fetal anemia than Amann et al. [1]. On the one hand, the 5.1% rate we observed could reflect a bias through the reference laboratory of CNRHP specialized in RBC alloimmunization in pregnancies. On the other hand, this rate is similar to the 6% reported in a French national survey, which collected data on IUT in the French referral fetal medicine centers [8].

Whilst the management of RBC alloimmunization is standardized worldwide, the management of suspected fetal anemia or fetal hydrops with no underlying immunization remains more complex. Prompt referral (within 24 h) is important to reduce the likelihood of demise before sampling, and to reduce the frequency of moribund hydrops, which impairs recovery from IUT. Beside fetal infection and fetomaternal hemorrhage cases that can be ruled out using basic biological examinations, the possibility to achieve a precise diagnosis relies on a systematic approach. The analysis of each case should rely on: an expert fetal sonography as rare cases of fetal anemia may be the consequence of a fetal organ hemorrhage or associated with specific fetal anomalies such as upper limb malformations in DBA or Fanconi anemia [19], maternal or parental investigation (family history of hematological disorders), and complete FBS. The role of FBS is central and could not be replaced by amniocentesis or parental sampling. One should be aware that some specific tests performed on fetal blood will be instructive only if they are performed prior to the first IUT. Besides, fetal anemia complicated with hydrops is actually a life-threatening condition for the fetus and IUT is the only treatment to

save it. These invasive procedures may allow the continuation of the pregnancy during the time needed to determine the etiology and the prognosis.

Our experience allowed us to establish the comprehensive diagnosis algorithm provided in Figure 2. We hope that this methodology will be accessible to any fetal medicine center and not only expert centers. Both the fetal reticulocyte count and blood smear represent the basis of the diagnostic approach. The reticulocyte count will allow to distinguish aregenerative anemia, involving impaired production of RBC precursors, and regenerative anemia, resulting from blood loss or hemolysis. The value of 150×10^9/L has been extrapolated from data established on historical studies on FBS [12]. The fetal blood smear is mostly useful in cases of regenerative (peripheral) anemia. However, it should be performed at the time of the first FBS and within the first 24 h because it is of value for diagnostics only on fresh blood and its analysis is no longer possible after the first IUT.

Among the aregenerative causes, i.e., central anemia, B19 parvovirus infection represents a leading cause that will be confirmed by a specific PCR analysis in amniotic fluid or fetal blood. Similarly, CMV infection should be systematically searched for. A very unusual cause is represented by congenital syphilis as observed in our case 1 [18]. When fetal infection has been ruled out, one should consider thalassemic syndromes and hemoglobinopathies. In this circumstance, the parental origin should be taken into account and the final diagnosis relies on hemoglobin electrophoresis and globin gene analysis in both the parents and the fetus [4, 20]. The most severe form of α-thalassemia is called Bart's disease. The absence of 4 copies of α-globin genes in a fetus causes severe anemia, leading to hydrops during fetal life. This autosomal recessive condition occurs at a higher frequency in some ethnic groups, such as Mediterranean, African, and South-East Asian populations. If fetal blood is taken by cordocentesis, Hb Bart's can be identified. When confirmed, parents should be informed of the poor prognosis and counseled about the 25% recurrence risk [21]. When all these causes have been ruled out, a dyserythropoiesis should be searched for. The CDAs belong to a group of rare hereditary disorders characterized by congenital anemia with failure in erythropoiesis. Patients usually present moderate to severe normocytic (CDA-II) to macrocytic (CDA-I) anemia with a mild regeneration and hemolytic features, including splenomegaly and jaundice. A CDA diagnosis relies on bone marrow film analysis and genetic testing. The prevalence in Europe is 0.24 per million [16, 22]. Similarly, DBA is a congenital erythroid aplasia. The transmission is autosomal dominant with variable penetrance. It is defined by an aregenerative, usually macrocytic anemia. Anemia can be associated with intrauterine growth retardation, fetal anomalies (craniofacial, anomalies of thumbs, cardiac or genitourinary malformations). Several cases of DBA associated with fetal hydrops have also been reported [15, 23].

The regenerative causes represent a heterogeneous group of pathologies. Within this group, inherited erythrocyte enzymatic deficiencies such as G6PD and PK deficiencies and ABO incompatibilities represent the most common etiologies [24–29]. The diagnosis of enzymopathies relies on a specific enzymatic activities test or genetic testing. ABO incompatibility will be searched for, depending on the parents' blood groups, and confirmed by search for anti-A or anti-B antibodies in the maternal blood and direct anti-globin test on fetal blood. Extremely rare alloimmune cases are possible if the fetus has inherited a low-frequency RBC antigen from the father. They can be searched by cross-matching the fetus and the mother or the father and the mother, and by performing the father's complete antigen assessment. Besides, RBC membrane disorders will be suspected at fetal blood smear and confirmed on EMA test and ektacytometry, which measures the deformability of RBC under shear rate and osmotic gradient, and genetic testing. Among this group, hereditary spherocytosis is the most frequent RBC membrane disorder in Europe and has been associated with hydrops [30]. Fetal DNA banking should also be systematically considered to allow further genetic investigation.

It should be noted that beside this diagnosis approach, and particularly in cases associated with fetal hydrops, the overall prognosis is not only linked to the etiology of the anemia and its treatment. As always in cases of hydrops, fetal tolerance and evolution of sonographic and Doppler signs will be decisive in the fetal and neonatal outcome. Whatever the perinatal outcome, all the information provided by the investigations previously described and which must be performed mandatorily at the time of the first FBS, will be essential to the parental counselling for following pregnancies.

Conclusion

IUTs for rare and unknown causes of fetal anemia represented 5.1% of all transfused fetuses and 3.9% of overall transfusions performed between 2010 and 2017 in our referral center. The prognosis differs markedly between different etiological groups. The underlying etiology re-

mains undetermined in up to 46% of cases. Current improvement in genetic testing possibly based on next-generation sequencing analysis will probably allow these cases to be characterized. However, complete workup of fetal features (fetal imaging, fetal hemogram, and biology) is mandatory to correctly interpret sequencing data and to provide adequate genetic counselling.

Statement of Ethics

Subjects gave their written informed consent. The study protocol was approved by the research institute's committee on human research.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

No financial support or funding was provided for this work.

Author Contributions

Dr. Emeline Maisonneuve and Dr. Imane Ben M'Barek carried out the analyses, drafted the initial manuscript, and approved the final manuscript as submitted. Dr. Anne Cortey, Dr. Stéphanie Friszer, Dr. Vanina Castaigne, Mrs. Pauline Thomas, and Dr. Françoise Pernot coordinated data collection, critically reviewed the manuscript, and approved the final manuscript as submitted. Dr. Thierry Leblanc, Prof. Lydie Da Costa, Dr. Agnès Mailloux, and Dr. Cécile Toly N'Dour designed the data collection instruments, critically reviewed and revised the manuscript, and approved the final manuscript as submitted. Prof. Jean-Marie Jouannic coordinated and supervised data collection, reviewed and revised the manuscript, and approved the final manuscript as submitted. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

References

1 Amann C, Geipel A, Müller A, Heep A, Ritgen J, Stressig R, et al. Fetal anemia of unknown cause – a diagnostic challenge. Ultraschall Med. 2011 Dec;32(S 02 Suppl 2):E134–40.
2 Zwiers C, van Kamp I, Oepkes D, Lopriore E. Intrauterine transfusion and non-invasive treatment options for hemolytic disease of the fetus and newborn – review on current management and outcome. Expert Rev Hematol. 2017 Apr;10(4):337–44.
3 Rouillac-Le Sciellour C, Sérazin V, Brossard Y, Oudin O, Le Van Kim C, Colin Y, et al. Noninvasive fetal RHD genotyping from maternal plasma. Use of a new developed Free DNA Fetal Kit RhD. Transfus Clin Biol. 2007 Dec;14(6):572–7.
4 Mari G, Deter RL, Carpenter RL, Rahman F, Zimmerman R, Moise KJ Jr, et al.; Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. N Engl J Med. 2000 Jan;342(1):9–14.
5 Lai K, Li S, Lin W, Yang D, Chen W, Li M, et al. Invasive prenatal diagnosis of α-thalassemia to control Hb Bart's hydrops fetalis syndrome: 15 years of experience. Arch Gynecol Obstet. 2018 Aug;298(2):307–11.
6 Kreger EM, Singer ST, Witt RG, Sweeters N, Lianoglou B, Lal A, et al. Favorable outcomes after in utero transfusion in fetuses with alpha thalassemia major: a case series and review of the literature. Prenat Diagn. 2016 Dec;36(13):1242–9.
7 Désilets V, Audibert F, Wilson R, Audibert F, Brock JA, Carroll J, et al.; Society of Obstetrician and Gynaecologists of Canada. Investigation and management of non-immune fetal hydrops. J Obstet Gynaecol Can. 2013 Oct;35(10):923–38.
8 Girault A, Friszer S, Maisonneuve E, Guilbaud L, Cortey A, Jouannic JM. [Intrauterine blood transfusion: status report of 4years of practice in France (2011-2014)]. J Gynecol Obstet Hum Reprod. 2017 Feb;46(2):119–24.
9 Zhang EG, Regan F, Layton M, Paramasivam G, Wyatt-Ashmead J, Roberts I, et al. Managing the difficult case of fetal anemia. J Matern Fetal Neonatal Med. 2011 Dec;24(12):1498–503.
10 Forestier F, Hohlfeld P, Vial Y, Olin V, Andreux JP, Tissot JD. Blood smears and prenatal diagnosis. Br J Haematol. 1996 Nov;95(2):278–80.
11 Da Costa L, Galimand J, Fenneteau O, Mohandas N. Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. Blood Rev. 2013 Jul;27(4):167–78.
12 Forestier F, Cox WL, Daffos F, Rainaut M. The assessment of fetal blood samples. Am J Obstet Gynecol. 1988 May;158(5):1184–8.
13 Forestier F, Daffos F, Rainaut M, Bruneau M, Trivin F. Blood chemistry of normal human fetuses at midtrimester of pregnancy. Pediatr Res. 1987 Jun;21(6):579–83.
14 Da Costa L, O'Donohue MF, van Dooijeweert B, Albrecht K, Unal S, Ramenghi U, et al. Molecular approaches to diagnose Diamond-Blackfan anemia: the EuroDBA experience. Eur J Med Genet. 2018;61(11):664–73.
15 Da Costa L, Chanoz-Poulard G, Simansour M, French M, Bouvier R, Prieur F, et al. First de novo mutation in RPS19 gene as the cause of hydrops fetalis in Diamond-Blackfan anemia. Am J Hematol. 2013 Feb;88(2):160.
16 Iolascon A, D'Agostaro G, Perrotta S, Izzo P, Tavano R, Miraglia del Giudice B. Congenital dyserythropoietic anemia type II: molecular basis and clinical aspects. Haematologica. 1996 Nov-Dec;81(6):543–59.
17 Friszer S, Maisonneuve E, Mace G, Castaigne V, Cortey A, Mailloux A, et al. Determination of the optimal timing of serial in utero transfusions in red blood cell alloimmuniation. Ultrasound Obstet Gynecol. 2015;46(5):600–5.
18 Macé G, Castaigne V, Trabbia A, Guigue V, Cynober E, Cortey A, et al. Fetal anemia as a signal of congenital syphilis. J Matern Fetal Neonatal Med. 2014 Sep;27(13):1375–7.
19 Bagby G. Recent advances in understanding hematopoiesis in Fanconi Anemia. F1000Res. 2018 Jan;7:105.
20 Karnpean R, Fucharoen G, Fucharoen S, Ratanasiri T. Fetal red blood cell parameters in thalassemia and hemoglobinopathies. Fetal Diagn Ther. 2013;34(3):166–71.
21 Jatavan P, Chattipakorn N, Tongsong T. Fetal hemoglobin Bart's hydrops fetalis: pathophysiology, prenatal diagnosis and possibility of intrauterine treatment. J Matern Fetal Neonatal Med. 2018 Apr;31(7):946–57.
22 Iolascon A, Esposito MR, Russo R. Clinical aspects and pathogenesis of congenital dyserythropoietic anemias: from morphology to molecular approach. Haematologica. 2012 Dec;97(12):1786–94.
23 Wlodarski MW, Da Costa L, O'Donohue MF, Gastou M, Karboul N, Montel-Lehry N, et al. Recurring mutations in RPL15 are linked to hydrops fetalis and treatment independence in Diamond-Blackfan anemia. Haematologica. 2018 Jun;103(6):949–58.

24 Gilsanz F, Vega MA, Gomez-Castillo E, Ruiz-Balda JA, Omenaca F. Fetal anaemia due to pyruvate kinase deficiency. Arch Dis Child. 1993;69(5 Spec No):523–4.
25 Adama van Scheltema PN, Zhang A, Ball LM, Steggerda SJ, van Wijk R, Fransen van de Putte DE, et al. Successful treatment of fetal hemolytic disease due to glucose phosphate isomerase deficiency (GPI) using repeated intrauterine transfusions: a case report. Clin Case Rep. 2015 Oct;3(10):862–5.
26 Masson P, Rigot A, Cécile W. [Hydrops fetalis and G-6-PD deficiency]. Arch Pediatr. 1995 Jun;2(6):541–4.
27 Ravindranath Y, Paglia DE, Warrier I, Valentine W, Nakatani M, Brockway RA. Glucose phosphate isomerase deficiency as a cause of hydrops fetalis. N Engl J Med. 1987 Jan; 316(5):258–61.
28 Grace RF, Bianchi P, van Beers EJ, Eber SW, Glader B, Yaish HM, et al. Clinical spectrum of pyruvate kinase deficiency: data from the Pyruvate Kinase Deficiency Natural History Study. Blood. 2018 May;131(20): 2183–92.
29 Suria AA, Nurasyikin Y, Adibah AG, Cheah FC, Leong CF. ABO incompatibility and glucose-6-phosphate dehydrogenase deficiency presenting as Hydrops Foetalis. Clin Ter. 2014;165(3):151–4.
30 Fuchs F, Michaux K, Rousseau C, Ovetchkine P, Audibert F. Syphilis infection: an uncommon etiology of infectious nonimmune fetal hydrops with anemia. Fetal Diagn Ther. 2016; 39(1):74–7.