Original article

Reduced fetal movement during pregnancy: Is the Kleihauer-Betke test really useful?

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A B S T R A C T

Introduction: Reduced fetal movement (rFM) is a frequent cause of consultation during the pregnancy and can reveal feto-maternal hemorrhage (FMH) that is sometimes responsible of severe fetal anemia. Our primary objective was to evaluate the contribution of the KBT in case of rFM. Our secondary objective was to compare it with ultrasound examination including peak systolic velocity of the middle cerebral artery (MCA-PSV) to predict neonatal anemia.
Materials and methods: We conducted a retrospective study from January 2016 to December 2017 at Armand-Trousseau Hospital in Paris. We analyzed all patients consulting for rFM from 18 to 41 weeks of gestation. We compared the performance of KBT and MCA-PSV to predict neonatal anemia (Hemoglobin at birth under 13.5 g/dL) and severe neonatal anemia (Hb < 10 g/dL).
Results: Among the 338 patients, 327 KBT (96.7%) were performed. KBT was found positive in three cases (0.9%). Only one neonate (0.3%) presented with severe anemia requiring a postnatal transfusion. MCA-PSV was performed in 166 cases (49.1%). KBT and MCA-PSV were significantly correlated with neonatal hemoglobin at birth. KBT was better than MCA-PSV to predict neonatal anemia, while MCA-PSV was better than KBT to predict moderate to severe anemia. The KBT and MCA-PSV Doppler had excellent sensitivity and predictive negative values (100%), but they had poor predictive positive values for severe neonatal anemia.
Conclusion: In case of decreased fetal movement, we suggest performing fetal cerebral Doppler. MCA-PSV could suffice in first approach. KBT may be performed if there is suspicion of fetal anemia in order to confirm FMH.

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Introduction

Reduced fetal movement (rFM) is a frequent cause of consultation during the pregnancy. Several studies have suggested that fetomaternal hemorrhage (FMH) could be only revealed by rFM [1–4]. Although severe FMH is rare, it represents a life-threatening condition for the fetus, as it could be responsible for acute fetal anemia, intrauterine death and/or severe neonatal anemia [1,4]. Fetal anemia could be suspected in case of sinusoidal heart rate pattern and when peak systolic velocity of the middle cerebral artery (MCA-PSV) is over 1.5 multiples of median (MoM) at Doppler examination [5,6]. To detect and quantify FMH, the Kleihauer-Betke test (KBT) is the standard method and the most widely used [7]. However, there are no recommendations about the realization of KBT and MCA-PSV Doppler within this context [8,9]. The primary objective of our study was thus to evaluate the performance of the KBT to detect FMH in case of rFM. Our secondary objective was to compare it with fetal MCA-PSV to predict adverse outcomes in relation with FMH.

Materials and methods

Study design

We conducted a retrospective study from January 1st, 2016 to December 31st, 2017 at Armand Trousseau Hospital in Paris. We reviewed all the cases of women with singleton pregnancies visiting the emergency ward for rFM from 18 to 41 weeks of gestation (WG) and 5 days. We excluded cases of trauma, maternal

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vaginal bleeding and red blood cell (RBC) alloimmunization. Cases with intrauterine fetal death not related to fetal hemorrhage were also excluded. In case of multiples consultations for this reason, we analyzed data of the last consultation. For each patient, the following obstetrical data were collected: maternal age, parity, gestational age, cardiotocography (CTG) pattern, ultrasound data, result of KBT, management, gestational age at delivery, neonate's hemoglobin [10] and need for transfusion. In particular, we noted if CTG was sinusoidal, because this pattern is known to be associated with severe neonatal anemia [6]. All the KBT were performed by the same referral immunohematology laboratory (CNRHP, the national reference center for perinatal hemobiology). The KBT is based on the differential resistance between fetal and adult hemoglobin to acid [10]. A maternal blood sampling is exposed to an acid solution, which discolors adult hemoglobin but not fetal hemoglobin. The fetal red cells appear dark pink in contrary to maternal red cells, allowing them to be countable under the microscope [7,11]. The KBT was considered positive if it was superior to 1 fetal RBC for 10,000 maternal RBC. All ultrasound examinations were performed by the on-call residents and revised by a senior physician in all cases. MCA-PSV measurements were standardized as previously described [12]. Two types of ultrasound equipment were used (Voluson E6 and Voluson 730 Expert, General Electrics, Zipf, Austria). Each MCA Doppler measurement was interpreted as multiples of median (MoM) for gestational age [5]. Neonate's Hb was systematically performed on the umbilical cord sampling at birth with a point-of-care pH Meter that measured blood gases (GEM premier 4000, Werfen®). Neonatal anemia was defined as neonate's Hb < 13.5 g/dL and severe neonatal anemia as Hb < 10.0 g/dL [13].

Ethics

The study has been approved by the committee on human research of the French College of Obstetricians and Gynecologists (CEROG, IRB: 2019-OBST-0102).

Statistical analysis

Data were reported as mean (±standard deviation) or median values (Interquartile range) and percentages. Sensitivity, specificity, predictive positive and negative values, Receiver Operating Characteristics (ROC) curves of KBT and MCA-PSV to predict neonatal anemia (Hb < 13.5 g/dL) and severe anemia (Hb < 10 g/dL) were calculated and compared. A Pearson correlation test was performed for quantitative variables with a parametric distribution (MCA-PSV measurement and Hb level on umbilical cord at birth). A Spearman correlation test was performed for non-parametric quantitative variable (KBT). Statistical analyses were performed with R software version 5.1.1. P values were used to estimate the strength of association with each parameter and statistical significance was set at p < 0.05.

Results

There were 7738 infants born from singleton pregnancies at Armand-Trousseau hospital during the 2-year-period. Among those cases, 348 (4,5%) pregnant women had consulted for rFM during pregnancy (Fig. 1). Ten cases were excluded because of associated maternal bleeding, uterine trauma, RBC alloimmunization or intrauterine fetal death not due to FMH. Maternal age was 32.5 (±4.9) years old. Parity was 0 (IQR 0–1). Rhesus D phenotype was negative for 37 patients (11%). The median gestational age at the time of rFM was 37WG (IQR: 30–39, Range: 19–41WG). Among the 338 cases included, 327 KBT (96.7%) were performed. KBT was not interpretable because of F-cells in five cases. KBT was found
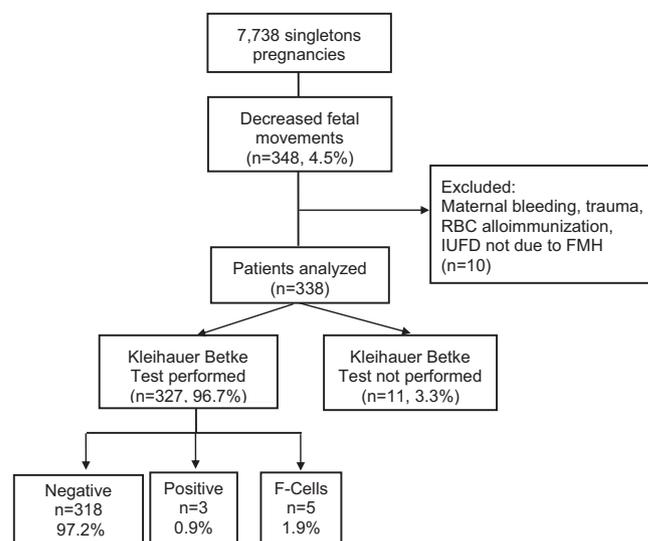

Fig. 1. Flowchart of the study.

positive in three (0.9%) cases (history below). One hundred and sixty-six (49.1%) measurements of the MCA-PSV were performed and gathered the criteria for quality in 81%. [12] The Doppler measurements were performed increasingly with gestational age (9.1%, 38.2%, 49.6%, 51.7% and 54.4% at < 24, 24–28, 28–37, 37−39 and ≥ 39WG respectively).

- Patient #1: A 27-year-old primigravida presented with rFM at 41WG. CTG was normal. Ultrasound was normal with a MCA-PSV at 61 cm/s corresponding to 0.94 MoM. The KBT found 91 fetal RBC/10,000 maternal RBC. An induction of labor was decided for the suspicion of FMH. Cesarean delivery was performed for arrest of cervical dilation. Neonatal blood sampling did not show anemia (13.5 g/dL).
- Patient #2: A 36-year-old primigravida was seen at 38WG because of rFM. CTG was sinusoidal (Fig. 2). KBT was very high at 551 fetal RBC/10,000 maternal RBC. Ultrasound demonstrated an elevated MCA-PSV at 94 cm/s (1.6 MoM). An emergency cesarean section was performed and neonatal hemoglobin was at 4.7 g/dL. The neonate was transfused in the first hour of life and the evolution was favorable. No etiology of the FMH was found.
- Patient #3: A 36-year-old, para 2, presented with rFM at 39WG. CTG showed reduced variability. KBT was at 153 fetal RBC/10,000 maternal RBC. Ultrasound was normal with a MCA-PSV at 50 cm/s (0.81 MoM). An emergency cesarean delivery was decided for FMH. Neonatal hemoglobin was 12.6 g/dL.

Fetal anemia was suspected on MCA-PSV measurements in two cases: patient #2 with a massive FMH associated with severe neonatal anemia (Hb 4.7 g/dL) and another patient whose ultrasound examination demonstrated an elevated MCA-PSV at 1.78 MoM but without neonatal anemia (Hb 14.2 g/dL). That case corresponded to a false positive of MCA-PSV. The CTG showed reduced variability with no acceleration and KBT was negative at 29WG. A cesarean section was decided for abnormal CTG and suspicion of fetal anemia.

CTG was sinusoidal only in the case of severe neonatal anemia. In total, we noted 9 cases of mild to moderate neonatal anemia and one severe neonatal anemia. Neonatal outcomes are reported in the Table 1. Among the 11 cases in which KBT was not performed, none had anemia at birth. The KBT and MCA-PSV Doppler had
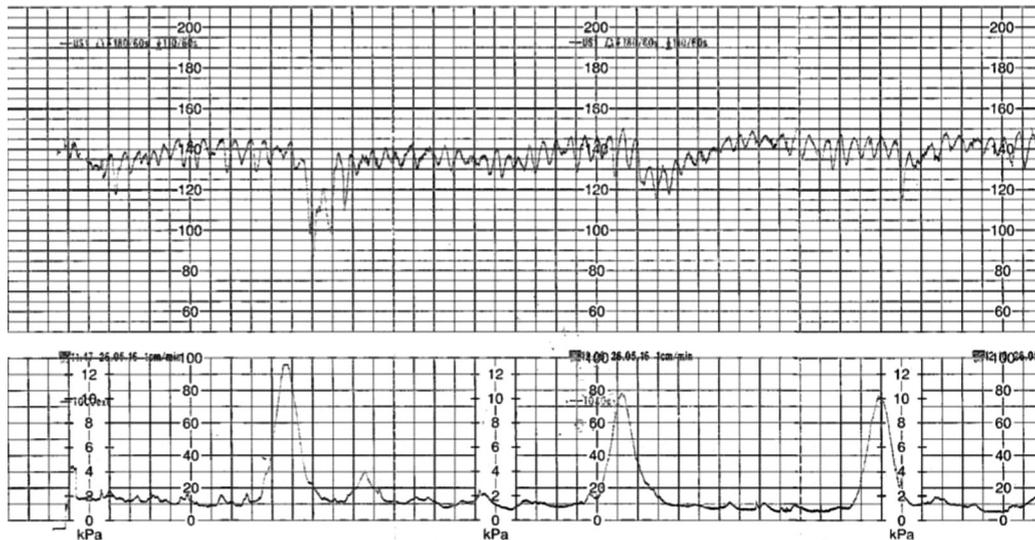
Fig. 2. Cardiotocography of patient #2.

Table 1
Neonatal outcomes.

	N	%
Live birth	338	100
Gestational age at delivery		
24–32	2	0.6
32−37	3	0.9
37−39	38	11.2
≥ 39	295	87.3
Neonatal anemia (Hb < 13.5 g/dL)	10	4.4
Severe neonatal anemia (Hb < 10 g/dL)	1	0.3
Need for transfusion	1	0.3

Discussion

Among 338 patients presenting rFM, three patients (0.9%) between 38 and 41WG had a positive KBT and only one neonate (0.3%) presented with severe anemia requiring emergency delivery and a postnatal transfusion. One hundred and sixty-six measurements of the MCA-PSV were performed (49.1%) and were pathologic in two cases. KBT was better than MCA-PSV to predict neonatal anemia, while MCA-PSV was better than KBT to predict severe anemia.

The main strength of the study relies on the detailed data on CTG, ultrasound examinations, KBT and neonatal hemoglobin levels. The second strength is represented by the analysis of the association between KBT, MCA-PSV and hemoglobin at birth within a homogeneous population receiving similar prenatal care and presenting rFM without any other pregnancy complications. However, the limitations of our study should be acknowledged. The first one is represented by the small number of cases with FMH, which is related to the low incidence of this complication. The second limit concerns the two FMH with Hb levels at 12.6 and 13.5 g/dL, which were not severe anemias. In these cases, the delivery was induced quickly because the gestational ages were 41 and 39WG and evolution of FMH is unpredictable. We do not know if a severe fetal anemia would have appeared in these 2 cases. Another limit is the variable time between MCA-PSV measurement

excellent sensitivity and predictive negative values (100%), but they had poor predictive positive values for severe neonatal anemia (Table 2). ROC curves demonstrated that KBT was better than MCA-PSV to predict neonatal anemia (respective areas under curve (AUC): 0.82 *versus* 0.73), while MCA-PSV was better than KBT to predict severe anemia (AUC: 0.75 *versus* 0.67). There was a significant correlation between MCA-PSV Doppler and Hb value at birth (r = −0.25, 95% confidence interval [CI] CI −0.42; −0.07, p = 0.006). (Fig. 3A) There was also a statistical correlation between Hb value at birth and KBT (r = −0.22, p = 0.0009) (Fig. 3B).

Table 2
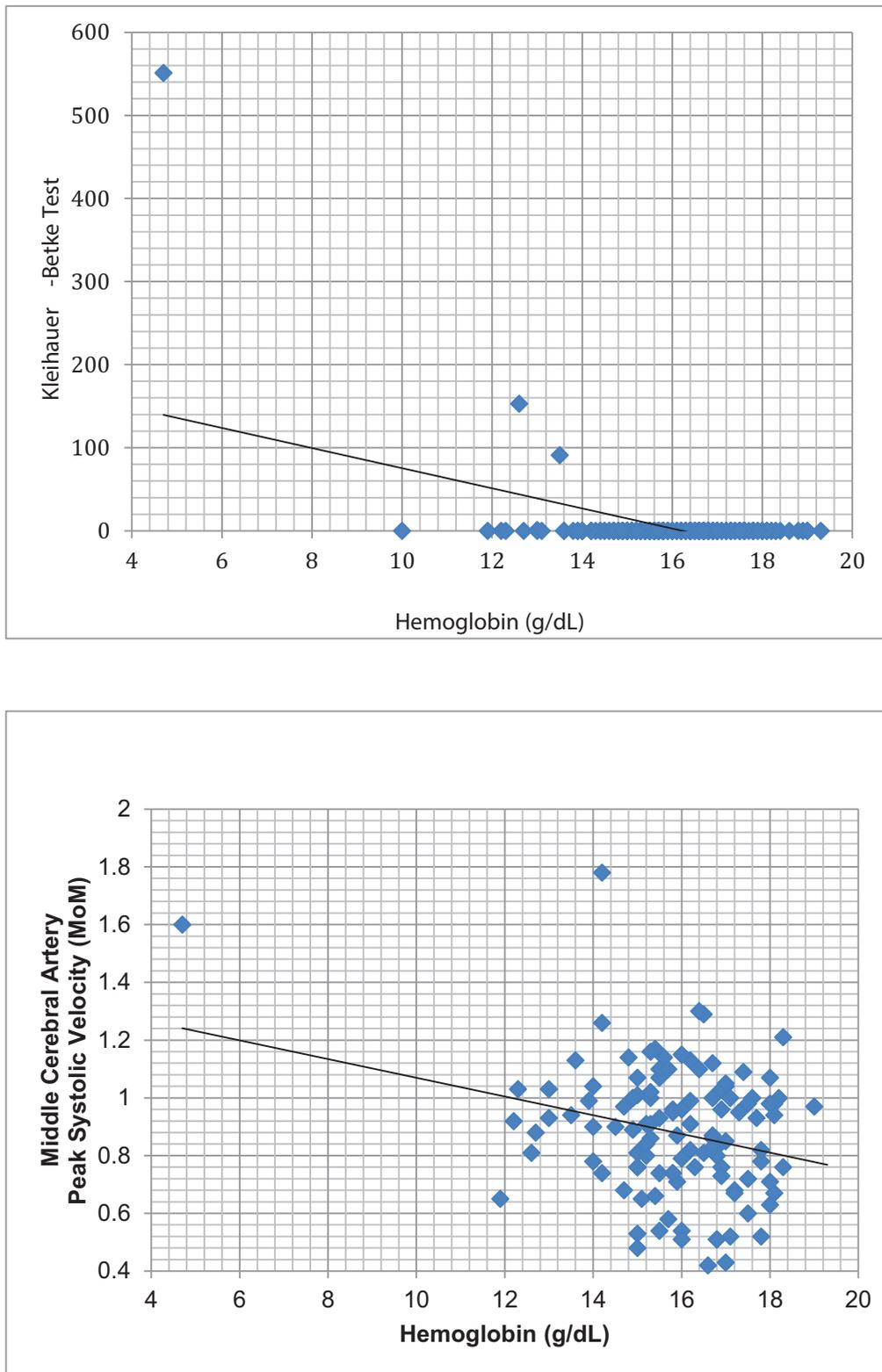Accuracy of CTG, Kleihauer-Betke test and MCA-PSV Doppler and to detect moderate to severe fetal anemia.

	Hb cut-off	Sensitivity	Specificity	PPV	PNV
Accuracy of CTG (N = 327)					
Severe neonatal anemia	< 10	1/1	326/326	1/1	326/326
N = 1		100%	100%	100%	100%
Accuracy of Kleihauer-Betke test (N = 327)					
Neonatal anemia	< 13.5	2/10	317/317	2/3	317/318
N = 10		20%	100%	66.7%	99.7%
Severe neonatal anemia	< 10	1/1	318/326	1/3	318/318
N = 1		100%	97.5%	33.3%	100%
Accuracy of MCA-PSV Doppler (N = 166)					
Neonatal anemia	< 13.5	1/10	155/156	1/2	156/164
N = 10		10%	99.4%	50%	95.1%
Severe neonatal anemia	< 10	1/1	164/165	1/2	164/164
N = 1		100%	99.4%	50%	100%

Hb: hemoglobin, CTG: cardiotocography, PPV: predictive positive value, PNV: predictive negative value, MCA-PSV: peak systolic velocity of the middle cerebral artery. Hb cut-off are expressed in grams/decililter.

Fig. 3. Correlation between prenatal tests and hemoglobin value at birth.

3a: Correlation between Kleihauer-Betke test and Hemoglobin value.

Kleihauer-Betke test is expressed in number of fetal RBC among 10,000 maternal RBC; r = −0.22, p = 0.0009.

3b: Correlation between MCA-PSV Doppler and hemoglobin value.

r = −0.25, p = 0.006.

and hemoglobin blood sampling at birth. In 45 cases (13%), MCA-PSV Doppler was performed at second trimester, *i.e.* 3–4 months before birth and the comparison is less appropriate than at third trimester. Only 49.1% of patients had a MCA-PSV measurement.

The Doppler measurements were performed increasingly with gestational age. This represents a major bias in our results.

The prenatal workup and management of rFM are heterogeneous as there are no French recommendations especially for the

technique used for the detection of FMH, *i.e.* KBT, fetal tracing and MCA Doppler measurements. Within this context, the Perinatal Society of Australia and New Zealand has recently published clinical practice guidelines for the management of pregnant women with decreased fetal movements [14]. They recommended performing KBT or flow cytometry test and a MCA Doppler assessment. As in France, the Royal College of Obstetricians and Gynecologists did not evoke the KBT or the MCA-PSV in their guidelines on rFM [9].

Over the two years of the study, we reported only one case of severe neonatal anemia associated with severe FMH. In this case, KBT, MCA-PSV Doppler and CTG were abnormal. If emergency delivery had not been performed, it would have led to perinatal death. Two other cases had FMH but without severe neonatal anemia. We can question about the interest of detecting these FMH without any signs of fetal anemia, if there are no consequences on the fetus and newborn. Indeed, as Huchet and Brossard showed in 1988, there are a few asymptomatic FMH discovered with the realization of systematic KBT in the third trimester of pregnancy and for whom, there are no fetal or neonatal consequences [15].

Sensibility and specificity of MCA-PSV and CTG to detect severe neonatal anemia are close to 100% although there is a low number of cases. Huissoud et al. studied the optimal cutoff values of quantitative FMH parameters calculated from ROC curves for the prediction of poor perinatal outcome [16]. The optimal threshold maximizing both sensitivity and specificity was a KBT of 250/10,000: sensitivity 100% (95% CI 76–100) and specificity 96% (95%CI 77–100). In their study on 54 cases of FMH, they performed only one MCA-PSV measurement. The interest of MCA-PSV in addition to the CTG could not be demonstrated by statistical analysis in our study, because there was only one case of severe anemia. However, in this case, both MCA-PSV and CTG were pathological and reinforced the suspicion of severe fetal anemia due to FMH. These tests were very important to decide to perform emergency cesarean section, to warn the neonatologists and to order RBC products for the neonate. Moreover, from a practical point of view, after a CTG lasting 30 min, MCA-PSV Doppler can be obtained and interpreted rapidly by trained physicians and residents (< 10 min). On the other hand, the time to send maternal blood to the laboratory and obtain the result of the KBT takes a couple of hours at least. Another limit of performing only the KBT is the occurrence of false negative KBT in case of negative Rhesus D patients with a recent prophylaxis of RhD alloimmunization and in case of ABO maternofetal incompatibility, because there is a hemolysis of the fetal RBC in the maternal circulation [17]. Furthermore, KBT can be not interpretable in case of F-cells. Indeed proportions of F-cells are increased in persons with several acquired and inherited disorders, such as sickle cell disease and thalassemia, that are associated with an increased percentage of Hb F [18].

Numerous studies validated the accuracy of MCA-PSV Doppler to predict fetal anemia, mainly for pregnancies followed-up for RBC allo-immunization [5,12,19]. Concerning FMH, some case reports found a good prediction of fetal hemoglobin by MCA-PSV [10,20–22]. Bellussi et al. showed in their review that MCA-PSV is a reliable indicator of severe fetal anemia in case of FMH [4]. In their series of 35 cases (including 15 cases with rFM), CTG upon admission was sinusoidal in 18 cases, nonreactive in 6, decelerative in 2 and tachycardic in one. MCA-PSV was abnormal in all cases but one. The authors suggested including fetal cerebral Doppler in the evaluation of patients with rFM, particularly in those cases with ambiguous results of biophysical testing. However, this measurement can be technically difficult at the end of pregnancy [19].

Finally, pathological examination of the placenta should be performed in case of FMH to search for intraplacental choriocarcinoma [23,24]. Another complication of FMH in the long run is RBC alloimmunization. We recommend to prescribe to the women presenting FMH an indirect antiglobulin test 6 months after delivery.

Conclusion

Fetomaternal hemorrhage is a rare complication in pregnancy. The only symptom that may be present is the mother's perception of a decrease in fetal movement. Considering data of literature and those of our study, we suggest including ultrasound and fetal cerebral Doppler in the evaluation of patients with decreased fetal movements. MCA-PSV and CTG could suffice in first approach to detect severe anemia. KBT may be performed if there is suspicion of fetal anemia in order to confirm FMH, especially in situations associated with premature delivery decisions.

Declaration of Competing Interest

The authors declare that they have no conflict of interests.

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