Maternal bleeding complications in pregnancies affected by red blood cell alloimmunization

Klara Beitzl

Medical University Vienna

Iris Holzer (✉ iris.holzer@meduniwien.ac.at)

Medical University Vienna <https://orcid.org/0000-0003-1739-7545>

Günther F. Körmöczy

Medical University Vienna

Antonia Valentina Hein

<https://orcid.org/0000-0002-9552-0468>

Judit Föster

Medical University Vienna <https://orcid.org/0000-0001-6371-122X>

Rudolf Seemann

Medical University Vienna

Johannes Ott

Medical University Vienna

Barbara Ulm

Medical University Vienna

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Abstract

Purpose

To investigate whether women with red blood cell (RBC) alloimmunization are more likely to experience bleeding complications during pregnancy or delivery than women without RBC alloimmunization.

Methods

Retrospective study involving all singleton pregnancies affected by RBC alloimmunization and without pre-existing maternal bleeding disorders or placenta previa, from 1 July 1999 to 30 June 2019 (“cases”). Only bleedings not related to invasive procedures (amnio- or cordocenteses) were included. Cases were compared to controls without RBC alloimmunization, matched for maternal age and body mass index, from the same tertiary referral center in Austria.

Results

130 cases were compared to 130 controls. Cases had significantly more previous pregnancies and miscarriages and their newborns had lower birthweight and were more often transferred to the intensive care unit than newborns of controls. 18/130 (13.8%) cases, compared to 8/130 (6.2%) controls experienced any bleeding during pregnancy or delivery ($p = 0.061$). Bleeding most often happened during the third trimester (cases: 4.6% vs. controls 0.8%, $p = 0.12$) and during or after delivery (cases: 7.7% vs. controls: 4.6%, $p = 0.168$). Binary logistic regression for the prediction of any bleeding complication during pregnancy, delivery or postpartum revealed immunization against RBC antigens as the only independent contributor ($p = 0.04$). Age, smoking, or previous obstetric history had no influence on the likelihood of maternal bleeding complications. Neither RBC antibody specificity nor titers were predictive of maternal bleeding during pregnancy or delivery.

Conclusion

Pregnancies affected by RBC alloimmunization are at increased risk of maternal bleeding complications during pregnancy and delivery.

Introduction

Maternal alloimmunization to fetal red blood cell (RBC) antigens, mostly concerning D, followed by K, c, and E, may result in severe fetal and neonatal anemia, and eventually hydrops and death, during pregnancy or delivery [1]. The incidence of RBC alloimmunization in pregnant women range from 0.4–2.7% [2]. The risk could be reduced by routine use of Rh D immunoglobulin postpartum from 16–2% and by routine antenatal prophylaxis further to 0.3% [3, 4].

As a result of the implementation of routinely performed early alloantibody screening, the use of accurate guidelines and the specification of tertiary referral centers for fetal therapy, severe fetal alloimmune hydrops with potentially lethal outcome a few decades ago has almost disappeared [5–7]. Still, the survival of fetuses with severe hydrops is significantly lower than that of anemic fetuses without hydrops. In 1981, the intravascular intrauterine transfusion (IUT) has been introduced which is considered a safe procedure that significantly improves the perinatal outcome in fetuses with severe anemia [8]. The procedure-related fetal loss rate of IUTs is approximately 2%, with higher loss rates at lower gestational ages [9–11].

Fetal and neonatal outcomes in RBC alloimmunization have substantially improved, but data on maternal complications in these pregnancies are scarce. In the majority of pregnancies with RBC alloimmunization, the origin of maternal sensitization is not clear, and most antigen-negative women with antigen-positive partners do not develop potentially harmful antibodies (AB), while others develop specific AB that remain at low levels throughout gestation. Pre- and perinatal hemorrhage may cause alloimmunization, or boosting of maternal AB production in sensitized pregnancies [1]. A “predisposition” to bleeding complications might explain why some women develop clinically significant RBC alloimmunization while others don’t. These bleedings would not necessarily cause relevant vaginal hemorrhage but could explain alloimmunization, hypothetically through transplacental micro-hemorrhage during gestation or increased feto-maternal transfusion during birth and placental abruption.

The aim of this study was to evaluate the prevalence of bleeding complications in women alloimmunized to fetal RBC antigens at AB titers sufficient to potentially affect the fetus and newborn, compared to women without these AB, during pregnancy and delivery. We hypothesized that RBC alloimmunized women were more likely to suffer from bleeding complications during pregnancy and postpartum compared to women without RBC AB.

Materials And Methods

In this retrospective cohort study, we included women who presented to the Department of Obstetrics and Gynecology at a tertiary referral center for RBC alloimmunization between July 1, 1999 and June 30, 2019.

We included all consecutive patients with viable pregnancies (at or above 12 + 0 gestational weeks, as confirmed by first trimester screening ultrasound) and with RBC AB and an AB titer ≥ 16 for anti-D and other non-D AB, or ≥ 4 for anti-Kell, respectively [12–14]. Exclusion criteria comprised AB titers below these specific thresholds; other AB not possibly affecting the offspring; non-invasive maternal blood testing results indicating an antigen negative fetus; birth of an antigen-negative child; and unknown pregnancy outcome, multiple pregnancies, and maternal age < 18 years. Furthermore, we excluded patients with pre-existing bleeding disorders (thalassemia, thrombophilia, factor V Leiden, protein S deficiency) and women with antithrombotic therapy or placenta previa. This resulted in a final study cohort of 130 pregnancies with RBC alloimmunization (Flowchart Fig. 1). Patients with RBC AB were

managed according to current guidelines [1], with ultrasound and Doppler assessment every 1–4 weeks, depending of the AB specificity and titer, gestational age, and history of previous affected pregnancies. When Doppler in the middle cerebral artery indicated fetal anemia or in the presence of fetal hydrops, cordocentesis and intrauterine transfusions were performed, according to standard protocols [1]. Delivery was planned at 36–37 week's gestation, after a last intrauterine transfusion, if required, at 34 weeks' gestation, for patients with RBC alloimmunization ("cases").

For group comparison, a control group of 130 pregnant women without RBC AB over the same 20-year period was randomly selected by matching cases to controls 1:1 applying matching criteria maternal age at presentation and pre-pregnancy body mass index ("controls").

The study protocol complies with the declaration of Helsinki and was approved by the Institutional Review Board of the Medical University of Vienna (institutional review board number 2217/2019). In retrospective studies neither written nor verbal informed consent is necessary according to the Ethics Committee of the Medical University of Vienna.

Parameters Analyzed

The primary endpoint of this investigation was the occurrence of any bleeding complications during pregnancy and delivery among cases (women with RBC AB) and matched controls (women without RBC AB) and the comparison between groups.

Bleeding that occurred in the setting of early abortion was not counted. Thus, only women who reached a gestational age $\geq 12 + 0$ weeks were included. Bleeding that occurred within one week of an invasive procedure such as amniocentesis, cordocentesis, or IUT, was presumed to be causally related and thus consequently not counted.

The following basic patient characteristics were collected: maternal age at first visit, body mass index (BMI), gravidity, parity, previous miscarriage and cesarean section, smoking during pregnancy and gestational age at first visit. We also assessed the occurrence of preterm premature rupture of membranes, stillbirth, gestational age at delivery, neonatal birth weight and transfer to neonatal intensive care unit (NICU). Data were acquired retrospectively using AKIM® software (SAP Software Solutions Austria, Vienna, Austria) and PIA Fetal Database software (GE-Viewpoint, Wessling, Germany), which is used as the basic perinatalogical database in our department.

In addition to maternal and fetal outcome parameters, AB titer at initial presentation, maximum AB titers, and gestational age at maximum titers were collected. The following maternal RBC AB antibodies resulted from our data research and were therefore enrolled: anti-D, anti-K, anti-c, anti-E, anti-M, anti-S, and anti-Fya. All AB specificities and titers were determined at the Department of Blood Group Serology and Transfusion Medicine, General Hospital of Vienna, Vienna, Austria according to ISO 9001 and ISO 15189 quality standards.

Routine blood group antigen typing was carried out by gel centrifugation technique using polyclonal or monoclonal Bio-Rad (Cressier, Switzerland) reagents and equipment, as described previously [15]. Anti-RBC antibody screening and identification was performed by IAT in gel matrix (Bio-Rad). Antibody titration was performed in tube technique with polyspecific anti-human globulin (Ortho Clinical Diagnostics, Neckargemünd, Germany).

Statistical Analysis

Continuous data are shown as mean $\pm$ standard deviation or median (minimum, maximum) and compared using Mann Whitney U-Test. Categorical parameters are presented as counts and percentages and analyzed using Chi-square test or Fisher’s exact test. Univariable binary regression models were used to assess the predictive potential of immunization on any bleeding events. For these regression models, odds ratios (OR) with 95% confidence intervals (95% CI) and the corresponding p-values are provided. Statistical significance was defined by two-sided *P*-values < 0.05. Statistical analyses were performed using SPSS 24.0 (IBM SPSS, USA).

Results

One-hundred-and-thirty women with RBC alloimmunization and 130 controls without RBC AB were included in the analysis. One-hundred-and-two women had anti-D AB (102/130, 78.5%), nine anti-E (6.9%), eight anti-c (6.2%), and seven anti-Kell (5.4%). Four women (3.1%) had other RBC AB (Table 1). The median RBC AB titer at the initial visit at our center was 64 (min-max: 0-4000), the median maximum AB titer encountered was 256 (min-max: 4-160000) at a median gestational age (GA) of 30.0 weeks (IQR: 23.8–33.0 weeks). Patient characteristics of cases and controls are summarized in Table 2. Women with RBC alloimmunization had significantly more previous pregnancies and deliveries than controls (*p* < 0.001), and they were significantly more likely to have had a previous miscarriage (*p* = 0.023).

Table 1
*Antibody distribution among
130 women with RBC
alloimmunization*

Antibody type	N	%
anti-D	102	78.5
anti-E	9	6.9
anti-K	7	5.4
anti-c	8	6.2
anti-M	1	0.8
anti-S	1	0.8
anti-Fya	2	1.5

Table 2
Patient characteristics for women with (“immunized”) and without red cell alloantibodies (“non-immunized”)

		Immunized	Non-immunized	p
N		130	130	-
Age (years)		31.3 ± 5.3	31.4 ± 5.3	0.877 ⁺
Pre-pregnancy BMI (kg/m ²)		24.9 ± 5.2	24.4 ± 5.7	0.518 ⁺
Primigravida	yes	5 (3.8)	41 (31.5)	< 0.001 [#]
	no	125 (96.2)	89 (68.4)	
Nullipara	Yes	10 (7.7)	45 (34.6)	< 0.001 [#]
	No	120 (92.3)	85 (65.4)	
Smoking during pregnancy		22 (16.9)	13 (10.0)	0.145 [#]
Previous cesarean section		40 (30.8)	30 (23.1)	0.208 [#]
Previous miscarriage		50 (38.5)	32 (24.6)	0.023 [#]
Gestational age at initial visit (completed weeks)		17.7 ± 7.5	17.5 ± 8.7	0.831 ⁺
Numerical parameters are provided as mean ± standard deviation, categorical parameters as numbers (frequencies); italic letters indicate statistical significance, ⁺ Mann Whitney U-Test, [#] Fisher’s exact test (2-sided)				

Women with RBC alloimmunization tended to have more overall bleeding complications during pregnancy and delivery, compared to controls (p = 0.061, Table 3). The rates of hemorrhage during the first (2.3% among cases vs. 1.5% among controls; p = 1) and second (0% vs. 1.5%; p = 0.498) trimesters did not differ between groups. Third trimester bleeding complications were slightly more common in women with RBC alloimmunization (4.6%) than in controls (0.8%; p = 0.12). Intra- and postpartum bleeding was again slightly more common among RBC alloimmunized women (7.7%) than among controls (4.6%; p = 0.168). Details of maternal bleeding complications are provided in Table 4. Details of outcomes are listed in Suppl. Table 1.

Table 3
*Bleeding complications during pregnancy and delivery, and outcome for women with
 ("immunized") and without red cell alloantibodies ("non-immunized")*

	Immunized	Non-immunized	p [#]
N	130	130	-
Any bleeding	18 (13.8)	8 (6.2)	0.061
Any bleeding during pregnancy	9 (6.9)	5 (3.8)	0.410
1st trimester bleeding	3 (2.3)	2 (1.5)	1.000
2nd trimester bleeding	0	2 (1.5)	0.498
3rd trimester bleeding	6 (4.6)	1 (0.8)	0.120
Intra- and postpartum bleeding	10 (7.7)	4 (4.6)	0.168
Categorical parameters are provided as numbers (frequencies); [#] Fisher's exact test (2-sided)			

Table 4
Details of maternal bleeding complications

Nr.	AB	Gestational age at time of bleeding (weeks + days)	Bleeding details	Estimated blood loss	Hb level before bleeding	Hb level after bleeding
Immunized						
1	anti-c	10 + 0	vaginal bleeding of unknown origin	n/a	n/a	13.1
2	anti-D	12 + 2	vaginal bleeding of unknown origin	n/a	n/a	14.3
3	anti-D	13 + 1	vaginal spotting of unknown origin, no hospital admission	n/a	12.8	12.5
4	anti-D	25 + 5	PROM and preterm labor	n/a	11.2	10.0
5	anti-D anti-C	31 + 3	severe intrapartum hemorrhage during c.s., preterm PROM, abruption of placenta	> 500mL	10	9.1
6	anti-D	33.+0	unknown origin, no hospital admission	n/a	n/a	n/a
7	anti-D	33 + 3	unknown origin, no hospital admission	n/a	n/a	n/a
8	anti-D	34 + 2	PPH, c.s.	2000mL	11.5	8.6
9	anti-D	34 + 6	PPH, c.s.	> 1000mL	11.9	11.2
10	anti-D	36 + 0	PPH, vaginal delivery	> 500mL	n/a	12.6
11	anti-D anti-C	37 + 1	PPH, c.s.	> 1000mL	8.2	6.9
12	anti-D	37 + 5	PPH, c.s.	> 1000mL	9.7	8.6

n/a,not applicable; AB, antibodies; Hb, hemoglobin; PROM, premature rupture of membranes; c.s., cesarean section;

PPH postpartum hemorrhage; RBC, red blood cells

Nr.	AB	Gestational age at time of bleeding (weeks + days)	Bleeding details	Estimated blood loss	Hb level before bleeding	Hb level after bleeding
13	anti-D anti-C	37 + 6	PPH, vaginal delivery	> 500mL	12.7	10.7
14	anti-D	37 + 6	Placental abruption bleeding, intrauterine fetal death	700mL	10.8	10.3
15	anti-D	38 + 3	PPH, vaginal delivery	> 1000ml	10.8	11.2
16	anti-D	38 + 6	vaginal bleeding, PROM	n/a	12.6	12.4
17	anti-K	39 + 2	PPH, vaginal delivery, uterine atony and placental retention, 4 RBC transfusions	> 500mL	12.5	6.8
18	anti-D	39 + 5	unknown origin	n/a	n/a	11.6
19	anti-D	40 + 0	Placental abruption	n/a	9.4	8.1
Non-immunized						
1	0	first trimester	vaginal bleeding of unknown origin, inpatient stay at external hospital	n/a	n/a	n/a
2	0	first trimester	vaginal spotting of unknown origin, no hospital admission	n/a	n/a	n/a
3	0	15 + 0	vaginal spotting of unknown origin, no hospital admission	n/a	n/a	n/a
4	0	22 + 2	vaginal bleeding, infection	n/a	10.6	10.3
5	0	28 + 0	vaginal bleeding, infection, PROM	n/a	13.4	13.1
6	0	34 + 0	PPH, after vaginal delivery, placental retention	> 1000mL	11.0	8.0
7	0	38 + 1	PPH, c.s.	> 1000mL	13.6	9.8

n/a, not applicable; AB, antibodies; Hb, hemoglobin; PROM, premature rupture of membranes; c.s., cesarean section;

PPH postpartum hemorrhage; RBC, red blood cells

Nr.	AB	Gestational age at time of bleeding (weeks + days)	Bleeding details	Estimated blood loss	Hb level before bleeding	Hb level after bleeding
8	0	39 + 2	PPH, vaginal delivery and cervical tear	800mL	11.7	10.2
9	0	41 + 5	PPH, c.s., uterine atony	> 1000mL	8.5	7.5
n/a,not applicable; AB, antibodies; Hb, hemoglobin; PROM, premature rupture of membranes; c.s., cesarean section;						
PPH postpartum hemorrhage; RBC, red blood cells						

In binary logistic regression, RBC alloimmunization and maternal bleeding complications during pregnancy and delivery were strongly associated (OR = 2.451; 95% CI 1.025–5.858). No association was found between maternal bleeding complications and maternal age, body mass index, gravidity, parity, smoking status, or previous cesarean section and miscarriage (Table 5). Neither the AB specificity (anti-D, anti-Kell, or other AB) nor AB titers were associated with maternal bleeding complications (Table 6).

Table 5
Binary logistic regression model for the prediction of any bleeding complications during pregnancy or delivery

		Any bleeding	No bleeding	OR	p
N		26	234	-	-
Age (years)		30.7 ± 5.1	31.4 ± 24.6	0.976 (0.903;1.054)	0.537
Pre-pregnancy BMI (kg/m ²)		24.9 ± 7.2	24.6 ± 5.3	1.010 (0.922;1.105)	0.837
Gravidity	1	1 (3.8)	45 (19.2)	ref	0.203
	2	10 (38.5)	67 (28.6)	6.716 (0.831;54.304)	
	≥ 3	15 (57.7)	122 (52.1)	5.533 (0.710;43.103)	
Parity	0	2 (7.7)	53 (22.6)	ref	0.238
	1	12 (46.2)	85 (36.3)	3.741 (0.805;17.377)	
	≥ 2	12 (46.2)	96 (41.0)	3.312 (0.714;15.360)	
Smoking during pregnancy		1 (3.8)	34 (14.5)	0.235 (0.031;1.794)	0.136
Previous Cesarean Section		6 (23.1)	64 (27.4)	0.797 (0.306;2.074)	0.642
Previous miscarriage		10 (38.5)	72 (30.8)	1.406 (0.609;3.249)	0.425
Immunization		18 (69.2)	112 (47.9)	2.451 (1.025;5.858)	0.044
Numerical parameters are provided as mean ± standard deviation, categorical parameters as numbers (frequencies); italic letters indicate statistical significance					
ref, reference					

Table 6

Binary logistic regression model for the prediction of any bleeding complications in immunized patients during pregnancy or delivery

	Any bleeding	No bleeding	OR	p
Antibody anti-D	15 (83.3)	87 (77.7)	ref	0.824
anti-K	1 (5.6)	6 (5.4)	0.967 (0.109;8.610)	0.976
others	2 (11.1)	19 (17.0)	0.611 (0.129;2.896)	0.534
Titer level at initial visit	64 (0-1024)	64 (0-4000)	0.999 (0.997–1.001)	0.406
Maximum titer	256 (4-16000)	256 (8-16000)	1 (1–1)	0.688
Numerical parameters are provided as median (min-max), categorical parameters as numbers (frequencies)				
ref, reference				
*Maternal age < 18 years; RBC antibody titers below the threshold; Other antibodies not possibly affecting the offspring; Non-invasive maternal blood testing results indicating an antigen negative fetus; Birth of an antigen-negative child; Unknown pregnancy outcome; Multiple pregnancies; Placenta previa; Maternal pre-existing bleeding disorders: thalassemia, thrombophilia, factor V Leiden, protein S deficiency; Current antithrombotic therapy				
*Controls without RBC antibodies, matched 1:1 to cases for maternal age and BMI; same exclusion criteria as for cases applied, except for AB and antigen-status of the fetus and newborn				

Discussion

We investigated the prevalence of maternal bleeding complications during pregnancy and delivery in women with RBC alloimmunization, compared to controls without RBC alloantibodies. Women with RBC alloimmunization had a higher overall prevalence of bleeding complications than controls. Higher rates of maternal hemorrhage in RBC alloimmunized cases, compared to controls, were independent of maternal age or previous obstetric history. Of note, bleedings that occurred within one week after an invasive procedure, such as amniocentesis or cordocentesis, were not counted for this study.

While the fetal risks and outcomes in RBC alloimmunization are well known [16], maternal complications in RBC alloimmunized women have not been studied so far. To the best of our knowledge, this investigation is the first to provide data on maternal bleeding complications in RBC alloimmunized women during pregnancy and delivery.

Despite substantial improvements in fetal medicine when implementing management according to Doppler assessment of middle cerebral artery as the threshold for fetal intervention [17] and gaining experience in IUTs over the last decades, adverse fetal or neonatal outcomes still exist. A large review of IUTs over 20 years in Sweden demonstrated a procedure-related complication rate of 4.9%, predominantly associated with technical difficulties in placing the needle into the right position. Ten patients from this

review were delivered by emergency cesarean section resulting in live born children, but four mothers lost their children in conjunction with the IUTs [17]. Other studies report a fetal loss rate between 0.9 and 4.9% [18]. Fetal distress during or after the procedure is the most serious complication that may result in emergency delivery with the risk of prematurity, neonatal asphyxia or even infant death [18]. Procedure-related risks are increased when invasive, in utero transfusion is instituted prior to gestational weeks 22 to treat severe early-onset fetal anemia [19]. Data on the long-term outcome of children after antenatal transfusion demonstrated a normal neurodevelopmental outcome in more than 95%, with the highest risk for neurodevelopmental impairment in the presence of fetal hydrops [20]. In our study cohort, no severe complications after invasive procedures such as cordocentesis or IUT occurred. Thus, no additional patients had to be excluded due to procedure-related complications.

Even simple prenatal invasive procedures and every fetal intervention carry a specific risk of any kind of complications, typically bleeding or infection, to the mother, while not providing any direct medical benefits to the mother herself [21]. A recent review on maternal complications following open and fetoscopic fetal surgeries demonstrated a maternal complication rate of 6.2% for fetoscopic and 20.9% for open fetal surgeries [21]. The rate of serious maternal complications was 1.7% in fetoscopic and 4.5% in open procedures and included severe infection, sepsis, lung edema, complete heart block, hemorrhage, placental abruption, maternal cardiac arrest, uterine rupture and caesarean hysterectomy [21]. A retrospective investigation on 187 pregnancies after fetal surgery revealed a prevalence of premature, preterm rupture of membranes of 44.4%, chorio-amniotic membrane separation in 36%, chorioamnionitis in 4.5%, vaginal bleeding in 6.7% and placental abruption in 6.2% of patients [22]. Maternal complications of pregnancies after fetal surgery for spina bifida were chorio-amniotic membrane separation in 15% and maternal pulmonary embolism, chorioamnionitis, maternal heart block and uterine rupture in 2.1%, 1.4%, 0.7% and 0.7%, respectively [23].

The present study focused on maternal bleeding complications during pregnancy and delivery without considering procedure-related complications of fetal interventions. Our data demonstrate an about doubled rate of any bleeding complications in women with RBC (13.8%) alloimmunization, compared to women without RBC AB (6.2%). For the rarity of the disease, our study cohort was rather large, yet not large enough to allow for a detailed, statistically sound discrimination between groups for bleeding rates, according to the time frames of every single trimester or only for the delivery to postpartum period. While, for example, six women with RBC AB had bleeding complications during the third trimester (6/130, 4.6%), only one women of the control group without RBC AB experienced bleeding during this period (1/130, 0.8%; $p = 0.12$). Based on these proportions, sample size requirements to detect differences in bleeding complications for the third trimester separately, at a power of 80%, calculates the need for 284 women in each group included. Enrollment of such a large patient cohort, even when bleeding complications are not uncommon in the control and in the case groups, is unlikely to be successful for a relatively rare disease such as RBC alloimmunization.

Multiple factors can cause bleeding complications during pregnancy or delivery that can happen in the first, second, and third trimester of pregnancy [24]. Vaginal bleeding in the first trimester is usually defined

as threatened abortion, eventually related to fetal genetic disorders or implantation difficulties. Bleeding that develops later than the beginning of the second trimester is often idiopathic, and the main risks associated with antepartum hemorrhage of unknown origin are preterm birth and low neonatal birth weight [25–28]. Severe postpartum hemorrhage is among the most common causes of severe, maternal life-threatening complications [29, 30]. In women with RBC AB, predisposition to ante- or peripartum bleedings may not only be considered a relatively frequent – maternal - pregnancy complication, but hypothetically also the origin of RBC alloimmunization, and boosting of the maternal immune response for subsequent pregnancies.

In a large Korean study, no difference was found between Rh positive and Rh negative primigravida women in terms of adverse pregnancy outcome, e.g. preeclampsia, placenta previa, placental abruption, postpartum hemorrhage and the need for uterine artery embolization [31]. That study, however, did not consider women with RBC AB and only first pregnancies were included. Interestingly, there are studies about a relationship of ABO blood groups and preeclampsia or pregnancy-induced hypertension. Recently published data of a large cohort study of 5.1 million unique persons provide evidence that pregnancy-induced hypertension was less common in blood groups A and AB, as compared to blood group O [32]. In contrary, other studies suggested that maternal A or AB blood types, but not B, were found at increased risk of preeclampsia compared with O type women [33] or that blood group O was significantly more common in early-onset and less common in late-onset preeclampsia [34]. However, data about the ABO blood groups and bleeding complications in pregnancy are lacking so far.

Study Strengths And Limitations

A major strength of this study is that it is, to the best of our knowledge, the first to describe maternal bleeding complications in the setting of maternal RBC alloimmunization. All consecutive women with RBC alloimmunization at a single tertiary referral center were eligible. For the analysis, we excluded women with pre-existing bleeding disorders, placenta previa, and cases with hemorrhage related to prenatal invasive procedures. Due to the retrospective study design, complete data sets about the length of hospitalization in case of maternal bleeding, the need for administration of RBC concentrates and hemoglobin values before and after the bleeding were not possible to retrieve. For comparison between groups, we chose to match women without RBC AB from the same center, matched for maternal age and body mass index, and with the same exclusion criteria as for women with RBC AB. Basic patient characteristics for previous obstetric history, namely parity, gravidity, and the rate of previous miscarriages, differed between the two study groups, which is considered a limiting factor of the present investigation. Multivariate regression analysis revealed, however, that these differences in patient characteristics had no significant influence on the risk of maternal bleeding complications.

Conclusion

Our data demonstrate a higher prevalence of bleeding complications during pregnancy and delivery in women with RBC alloimmunization, compared to women without RBC AB. These bleeding complications

were not related to antenatal invasive procedures, placenta previa, or pre-existing maternal bleeding disorders. Most bleedings among women with RBC alloimmunization happened during the third trimester or during and after delivery. Maternal age, body mass index, smoking status, and previous obstetric history such as previous miscarriage or cesarean section were not associated with a higher likelihood of maternal bleeding complications. In this study, comparing matched cohorts of 130 women with RBC alloimmunization and 130 women without RBC AB, maternal alloimmunization to RBC antigens was an independent predictor of maternal bleeding complications.

Declarations

Funding: BU received funding as a Short Time Advisory Board Member of Momenta Pharmaceuticals. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. All other authors: No funding. Open access funding provided by Medical University of Vienna.

Conflicts of interest/Competing interests: The authors declare no conflict of interest.

Availability of data and material: Available upon request.

Code availability: Not applicable.

Ethics approval: The study complied with the principles outlined in the Helsinki Declaration of 1975, as revised in 2013, and was approved by the institutional review board of the Ethics Committee at the Medical University of Vienna (EK Number 2217/2019).

Consent to participate: In retrospective studies, neither written nor verbal informed consent is required according to the Ethics Committee of the Medical University of Vienna.

Consent for publication :As above.

Authors' contributions:

KB: Project development, Data analysis, Manuscript writing and editing.

IH: Data analysis, Manuscript writing and editing.

AVH: Data collection and management, Manuscript editing.

JF: Data collection and management, Manuscript editing.

GK: Data analysis, Manuscript writing and editing.

RS: Data analysis, Manuscript writing and editing.

JO: Protocol development, Data analysis, Manuscript writing and editing.

BU: Project development, Protocol development, Data analysis, Manuscript writing and editing.

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Figures

Figure 1

Flowchart of patient inclusion *Maternal age <18 years; RBC antibody titers below the threshold; Other antibodies not possibly affecting the offspring; Non-invasive maternal blood testing results indicating an antigen negative fetus; Birth of an antigen-negative child; Unknown pregnancy outcome; Multiple pregnancies; Placenta previa; Maternal pre-existing bleeding disorders: thalassemia, thrombophilia, factor V Leiden, protein S deficiency; Current antithrombotic therapy +Controls without RBC antibodies, matched 1:1 to cases for maternal age and BMI; same exclusion criteria as for cases applied, except for AB and antigen-status of the fetus and newborn

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