POSITIVE OUTCOME IN A PREGNANCY WITH ANTI-KELL ALLOIMMUNIZATION TREATED WITH INTRAVENOUS IMMUNGLOBULIN AND PLASMA EXCHANGE DESPITE PERSISTENCE OF HIGH TITRE ANTIBODY: A CASE REPORT

Abstract

Background: Few reports have been published of the current clinical management of anti-Kell alloimmunization in pregnancy; its low frequency of occurrence means that the few long series published have covered an extensive time period in which different treatment approaches have overlapped. The objective of the present paper is to present our experience in the clinical management of a pregnant woman who was positive for the anti-Kell antibody.

Materials and Methods: The laboratory follow-up included the weekly measurement of the antibody titre, and identification of the paternal and fetal genotype. The clinical management included PEx and IVIG administration. Obstetric monitoring included ultrasonographic monitoring of the fetus.

Case Description: We report a case of anti-Kell alloimmunization with high antibody titre at first observation. Testing for fetal DNA circulating in maternal blood confirmed positivity for KELL1 gene. The patient underwent three sessions of PEx before 18 weeks, followed by weekly IVIG infusion, which continued until 23–27 weeks of pregnancy. Anti-Kell titres were measured before and after each PEx session.

The patient had no need for IUBT and was delivered by cesarean section at 34 weeks of gestational age. Pregnancy resulted in a live birth with a mild HDFN.

Discussion: HDFN is a potentially lethal complication of alloimmunization, and IUBT is the standard treatment for severe fetal anemia. PEx and IVIG are two alternative treatment modalities described in the literature to avoid or postpone the need for IUBT transfusion. In the case reported, despite any substantial change in antibody titre, pregnancy resulted in a live birth. This result suggests that the use of PEx and IVIG in alloimmunization during pregnancy could be an effective treatment strategy.

Key Words:

Anti-Kell, Hemolytic Disease of Fetus and Newborn, Plasma Exchange, Pregnancy.

Background

Kell blood-group system consists of 23 antigens allocated on a transmembrane protein (MW 93KD) encoded by a single gene located on chromosome 7 (7q33). Kell antigens are expressed only by erythroid progenitor cells and mature erythroid cells. Kell blood-group system is considered relevant in transfusion medicine because anti-Kell can both life-threatening PTHR and severe HDFN [1-3].

It seems interesting to note how clinical and laboratory findings in HDFN mediated by anti-Kell antibodies differs in several ways respect to classical HDFN mediated by anti-D antibodies. In anti-Kell HDFN, fetuses have very low hemoglobin concentration with lower numbers of circulating reticulocytes and normoblasts, moreover the concentrations of bilirubin in amniotic fluid and in fetal or neonatal serum are relatively low. Moreover, the titre of anti-Kell antibodies in maternal serum correlates poorly with the degree of fetal anemia. These observations suggest that in Kell alloimmunization fetal anemia is caused by the suppression of erythropoiesis, in addition to hemolysis [4-7].

In women with allo-immunization, in the intent to prevent the insurgence of a HDFN, PEx in addition to IVIG administration are sometimes used with the aim of mitigating the disease and to prevent the need of IUBT. The ASFA guidelines suggest that allo-immunization in pregnancy is a recognized indication of PEx (with a category III indication), on the basis of a risk assessment performed for each individual case [8-10].

In this paper we report a case of a pregnant woman presenting a high titre anti-Kell antibody against who had a previous normal pregnancy. We describe the protocol used for management, antibody titration, fetal monitoring results, and fetal/neonatal outcomes.

Laboratory Methods

For routine immunohematology analysis in our laboratory adopted a fully automated gel test (DG Gel) supplied by Grifols and for ABO grouping (Forward and reverse group), D typing, Rh and Kell adopted fully automated system by Neo Iris Immucor.

Our extended serological typing profiles include the characterization for ABO, C, E, c, e, K, k, Kpa, Kpb, Fya, Fyb, Jka, Jkb, M, N, S, s, Lua, Lub, P1, Xga, Lea, Leb, using a fully automated gel test method (DG Gel) supplied by Grifols.

For genotyping, we adopted the microarray-based Immucor BioArray HEA v1.2 BeadChip kit supplied by Werfen that involves characterization for C, E, c, e, V, VS, K, k, Kpa, Kpb,

Jsa, Jsb, Fya, Fyb, Fyb-weak, Fy-null, Jka, Jkb, M, N, S, s, U-var, U-neg, Lua, Lub, Dia, Dib, Doa, Dob, Hy, Joa, Coa, Cob, Sc1, Sc2, LWa, LWb, U^{var}, *P and Red Blood Cells typing : ID CORE-XT- BLOOD CHIP - GRIFOLS.*
Laboratory methods have been validated before routine utilization.

Case Description

SM, a 31-year-old Caucasian woman, was referred to our centre on December 22th, 2022, to evaluate the positivity of the IAT, occasionally encountered during a routine pregnancy monitoring carried out at an external laboratory.
Her previous medical history reported a nephrectomy for polycystic kidney and hypothyroidism on hormone replacement therapy. No history of blood transfusion was recorded.
Obstetric history reported a previous pregnancy in 2020. The first pregnancy was uncomplicated and delivered at 38 weeks of gestation by cesarean section, she had a healthy male newborn (blood type: A RhD positive, CCee Kk, DAT negative), with no evidence of anemia or jaundice.
Actually, SM was on the 12th week of gestation, blood type A RhD positive, Ccee kk, DAT negative, IAT positive for presence of an anti-Kell antibody with titre 1024. Her husband: PFe, Caucasian, age 38, A RhD positive CCee Kk. In both subjects extensive red blood cell phenotyping and genotyping were performed, (Table I). Moreover, a molecular analysis of the fetal DNA in maternal blood was performed at Sanguis Diagnostek Amsterdam, showing, on January 12th, 2023, that the fetus was K positive. Upon referral to our centre for management of her second pregnancy at 12 weeks of gestational age, in a first phase we performed a policy of watchful waiting, by monitoring the antibody titre on a weekly basis, ensuring that a portion of the maternal plasma is frozen for future evaluations. From a gestational age of 18 weeks, in an attempt to prevent the onset of HDFN, it was agreed with the patient to carry out a treatment based on the execution of a PEx procedure once a week in association with the administration of IVIG. PEx sessions were performed using a Spectra Optia apheresis system (Terumo BCT, Lakewood, CO, USA), by processing 1.5 plasma volumes in each session and using 5% albumin in normal saline, as a replacement fluid. Usually, the anti-Kell titre decreases by one dilution after a single PEx session. Weekly IVIG was administered, immediately after the second PEx session, at a dose of 1 g. per kg of body weight. From gestational age of 26 weeks Pex treatment was stopped due to SARS.CoV19 swab positivity and weekly 1 g/Kg administration continued unti

delivery. Moreover, evaluation of PSV of MCA, with fetal hemoglobin extimation was performed (Table II).

The fetus did not develop signs of fetal hydrops throughout the follow-up period, none IUBT procedure was performed. The titre of anti-K fluctuated throughout the pregnancy from 1024 to 256, reaching 512 at the time of delivery (Figure 1).

The mother delivered by elective caesarean section on day May 15th, 2023, at 34 weeks of gestation. Apgar's score was 9/10/10 at 1’5’10’minutes. PFr, a male newborn weighed 2,730 g, and his Hct was 36%. The forward grouping of the cord blood sample showed that blood group A RhD positive, IAT positive due to passively transmitted maternal anti-K IgG antibody, DAT was positive (++++) for IgG; surprisingly, phenotyping for the K antigen was negative, thus a genotyping was performed, confirming positivity for Kell1. The newborn was jaundiced and required double phototherapy, but did not require any blood transfusion. He was discharged at the age of 10 days in good condition. Repeated investigations at four weeks and six 6 months after delivery confirmed the infant's blood group was A Rh(D) positive, Ccee, Kk, antibody screening performed on infant plasma and DAT were negative.

Discussion

PEx and IVIG have been used as immunomodulatory measures in many conditions. Several case reports, case series, and case–control studies support the effectiveness and safety of their use, separately or in combination, in patients at risk of early HDFN to delay or avoid the need for IUBT. In women with clinically significant red cell alloantibodies, titration of alloantibodies during pregnancy is recommended to allow physicians to identify pregnancies having the highest risk of fetal anemia. A titre of 6 (or a rising titre) is usually considered clinically significant and indicates the need for more frequent clinical or imaging fetal monitoring. An exception is anti-K, where the risk of HDFN is considered high at any titre. After identification of a fetal maternal alloimmunization and the need for frequent monitoring is established during pregnancy antibody titration is frequently monitored [11-13].

One of the essential findings among our patient was that clinical response was achieved despite the lack of a significant drop in antibody titre. In other studies, antibody titre dropped significantly after apheresis and remained low during IVIG therapy. Anti-D and

anti-K are both IgG antibodies recognized to have the potential for causing severe HDFN. However anti-K has a unique suppressive effect on fetal erythroid progenitors, in addition to antibody-mediated hemolysis. Therefore, in anti-Kell HDFN the fetal anemia is often much more marked than what is suggested by the fetal bilirubin values [14,15].

Patients may require apheresis treatment during pregnancy for many indications, and the procedure is associated with a high safety profile. However, a group of common complications may be encountered by all patients undergoing PEx, including central venous access complications (e.g., thrombosis, bleeding, and infection), citrate toxicity (presenting with hypocalcemia), arterial hypotension (mostly by vasovagal mechanism), and transfusion reactions (when plasma is used as replacement fluid). The risks of these complications are similar in pregnant women, with a higher risk of hypotension and resultant fetal distress [16,17].

The case described in this report presented a further point of interest because, at birth, the newborn resulted Kell negative at routine serological assay, but genotyping confirmed the presence of both Kell1 and Kell2 gene, therefore predicting a Kell/Cellan phenotype. This discrepancy was resolved four weeks after birth, thus hypothesizing a masking of the antigen by the high dose maternal IVIg treatment during pregnancy.

Conclusion : Based on the available literature, PEx and IVIG are effective measures to delay the need for IUBT in pregnant women with alloimmunization at risk of early HDFN. However, the published regimens are widely variable, and further studies are required to determine the most effective strategy. Nevertheless, a successful pregnancy may be achieved despite persistently a high antibody titre.

Abbreviations

- ASFA: American Society for Apheresis
- HDFN: Hemolytic Disease of Fetus and Newborn
- DAT: Direct Antiglobulin Test
- IAT: Indirect Antiglobulin Test
- IUBT: Intra Uterine Blood Transfusion
- IVIG: Intra Venous Immune Globulin
- MCA: Median Cerebral Artery
- MW: Molecular Weight
- PEx: Plasma Exchange
- PSV: Peak Systolic Velocity
- PTHR: Post Transfusion Hemolytic Reactions

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Table I: Serotyping and Genotyping for SM (mother), PFe (father) and PFr (newborn)

	SM (mother)*	PFe (father)*	PFr (newborn)**
ABO	A	A	A
RhD	Positive	Positive	Positive
RhCE	Ccee	CCee	Ccee
Kell	kk	Kk	Kk
Penney	Kpa-Kpb+	Kpa-Kpb+	Kpa-Kpb+
Sutter	Jsa-Jsb+	Jsa-Jsb+	Jsa-Jsb+
MNSs	M+N-S+s+	M+N+S-s+	M+N+S+s+
Kidd	Jka-Jkb+	Jka-Jkb+	Jka-Jkb+
Duffy	Fya+Fyb+	Fya+Fyb-	Fya+Fyb-
Scianna	Sc1+Sc2-	Sc1+Sc2-	not tested
Lewis	LWa+LWb-	LWa-LWb+	not tested
Lutheran	Lua-Lub+	Lua-Lub+	Lua-Lub+
Dombrok	Doa+Dob+	Doa-Dob+	not tested
Colton	Coa+Cob-	Coa+Cob-	Coa+Cob-
Diego	Dia-Dib+	Dia-Dib+	Dia-Dib+
Cartwright	not tested	not tested	Yta+Ytb-

*) Serotyping and Genotyping were performed at first observation in Dell'Angelo Hospital in Mestre. **) Serotyping and Genotyping were performed at birth in the University Hospital in Padua

Table II: Titre of anti-Kell antibody and fetal hemoglobin

Date	Anti-Kell titration*	Fetal Hb g/L**
12/22/22	1024	Not tested
12/27/22	1024	Not tested
01/05/23	512	Not tested
01/15/23	512	Not tested
→ 01/23/23	1024	99
01/30/23	512	118
02/09/23	512	114
02/16/23	256	118
02/23/23	256	118
02/27/23	256	126
03/02/23	256	115
03/09/23	256	107
03/16/23	512	109
03/30/23	512	124
04/06/23	512	127
04/13/23	1024	104
04/20/23	1024	132
04/27/23	512	133
05/04/23	512	129

*) Anti-Kell titer evaluation was performed before PEx sessions **) fetal hemoglobin was evaluated considering the flow velocity in MCA.

The red arrow indicates the date of the first PEx procedure

Figure 1: Anti-Kell antibody titre before and after each PEx procedure