CASE OF D-ANTIGEN BLOCKING IN A NEWBORN WITH SEVERE HDFN

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Background

Antigen blocking is a rare phenomenon caused by maternal IgG antibodies (ab) saturating newborn red blood cell (RBC) antigens. This may lead to false negative (-) typing results when using monoclonal IgM reagents. We report a case of Rhesus (Rh) D-antigen blocking in a newborn with a severe HDFN caused by high titre maternal (G III, inadequate RhD prophylaxis at G II) anti-D- (4096), beside anti-Jka- (64) and anti-C-ab (2) (**Table 1.**).

After early delivery at Kantonsspital Graubünden (week 36 + 0) the newborn needed top-up transfusions (ccddee, Jka-). Initially, the newborn was typed Ccddee, Jka+ and the direct antiglobulin test (DAT) was strongly positive (IgG 4+, C3d 3+). The eluate was specific for anti-D-, anti-Jk^a- and anti-C-ab (**Table 2.**).

Methods

To elucidate the discrepancy between the declared Rh phenotype (Ccddee) and eluate specificity (anti-D), a sample (newborn EDTA heel blood) was sent to our reference laboratory in Zurich. Pheno- and genotype analysis were performed by using standard techniques including two different saline reactive anti-D antisera (Grifols, CH) and commercially available PCR-SSP kits (inno-train, DE). Newborn's Rh phenotype was reevaluated serologically after dissociating the maternal IgG ab from the RBC by EGA treatment (EDTA Glycine-Acid Kit, Immucor, DE). DAT was performed by using a polyspecific anti-human globulin card (BioRad, CH) before and after the EGA treatment.

Results

Untreated RBC were typed as RhD-, and an observed mixed-field (MF) reaction with anti-C was consistent with the top-up transfusion directly after birth (**Figure 1a**). Three consecutive EGA treatments revealed a second MF agglutination with anti-D, predicting CcD.ee beside a ccddee RBC population (**Figure 1b**). The initially strongly positive DAT (3+) decreased markedly after EGA treatment (1+), as expected. A subsequent genotyping confirmed the serological typing as CcDdee, Jka+ (**Table 3.**).

Due to prolonged anemia the newborn received a total of three top-up transfusions directly after birth (Hb 64 g/L), on day 9 (Hb 65 g/L) and day 28 (Hb 84 g/L). Additionally, intensive phototherapy and O_2-therapy were given to treat hyperbilirubinemia and low saturation levels.

Table 1. Mother's antibody titres during pregnancy in Kantonsspital Graubünden.

Antibody	WG 30	WG 36
Anti-D	64	4096
Anti-Jk^a	4	64
Anti-C	1	2

Abbr. WG: week of gestation

Table 2. Initial immunohematological parameters of the newborn in Kantonspital Graubünden.

Parameter	Newborn
Blood group	O RhD neg
Rhesus phenotype	Ccddee, r'r', K-
DAT monospecific	IgG 4+, C3d 3+
Antigen determination	Jka+
Antibodies detectable in serum / eluate	Anti-D (4+ / 4+), Anti-C (+/-1) Anti-Jka (3+ / 3+)

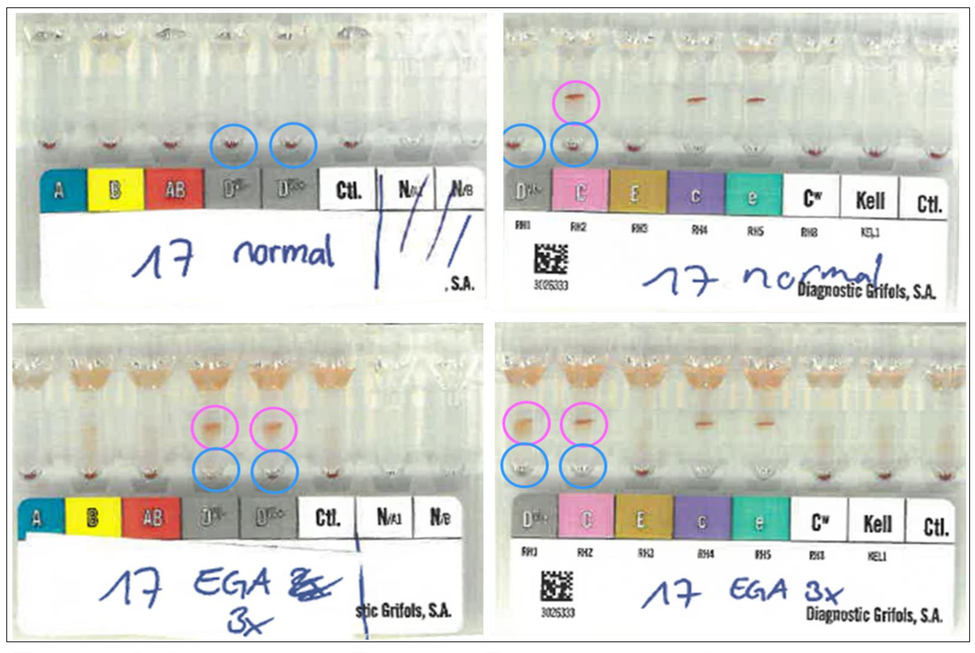

Table 3. Newborn Rhesus D typing results after birth.

Method	Untreated RBC	EGA-treated RBC
Gel card DAT IgG (reaction strength) untreated RBC	RhD neg 3+	MF with Anti-RhD 1+
	ISBT	**Predicted phenotype**
Genotyping	*RHD*01* | . *RHCE*01* | *RHCE*02*	CcD.ee

Serological results of pre-transfused newborn's RBC before and after EGA-treatment. Abbr.: RBC: red blood cell, EGA: EDTA Glycine-Acid Kit; RhD: Rhesus D; MF: mixed-field

Figure 1. Newborn's Rhesus D typing results after birth.
Serological results of pre-transfused newborn's RBC **a)** before and **b)** after EGA-treatment. Untreated RBC: RhD-, EGA-treated RBC: mixed-field with RhD. Newborn's phenotype (pink), Mother's phenotype (blue). Abbr.: EGA: EDTA Glycine-Acid Kit

Conclusion

We present a case of D-antigen blocking by maternal Anti-D in a newborn initially mistyped as RhD-. After dissociation of maternal high titre IgG by EGA the RhD+ phenotype of newborn RBC became detectable, which later was confirmed by genotyping. **Antigen blocking should be suspected in cases with severe fetal anemia in the presence of high titre maternal ab and a strongly positive DAT of newborn RBC which are not reacting with the corresponding IgM antiserum.**

References

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Abstract