German Red Cross Blood Service West

Deutsches Rotes Kreuz
DRK-Blutspendedienst West

A Case of Anti-CD36 induced NAIT in an Arabian Newborn and CD36 Donor Screening

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Background

CD36 isoantibodies have been reported to induce neonatal alloimmune thrombocytopenia (NAIT) especially in eastern Asian populations where CD36 deficiency is rather frequent with 2 to 11%. However, transfusion of compatible CD36 negative platelets to affected neonates is challenging when CD36 negative donors hardly can be found within the domestic donor population in central Europe.

Aim

We describe the successful platelet transfusion strategy in CD36 isoantibody induced NAIT in a neonate of Arabian ancestry and the CD36 screening approach in Arabian individuals.

Patients, Donors, and Methods

NAIT was suspected in a neonate of an Arabian family that was born with 27 platelets/nl without bleeding.

1328 volunteer blood donors mainly of Arabian and Iranian origin presenting at regular services of the German Red Cross Blood Service West were recruited for CD36 screening. All Individuals had given informed consent and the study was approved by the ethics committee of the Medical Chamber (Mainz, Germany).

Platelet antibody testing was performed by Luminex PAKLx (Immucor Lifecodes; Dreieich, Germany), MAIPA and flow cytometry. Screening for the presence of platelet CD36 (pCD36) was performed by flow cytometry on EDTA anti-coagulated peripheral blood with FITC labelled anti CD36 moab clone FA6-152 and PE labelled CD42b moab clone HIP1. Platelet CD36 deficient samples were subjected to genomic DNA sequencing.

Results

The maternal serum was positive with platelets from each donor in flow cytometry (fig. 1a). No GPIIb/IIIa, Ia/IIa, Ib/IX or CD109 specific antibodies were detected within the maternal serum by indirect MAIPA with donor or paternal platelets, nor with Luminex PAKLx. However, PAKLx identified **a rare anti-CD36.**

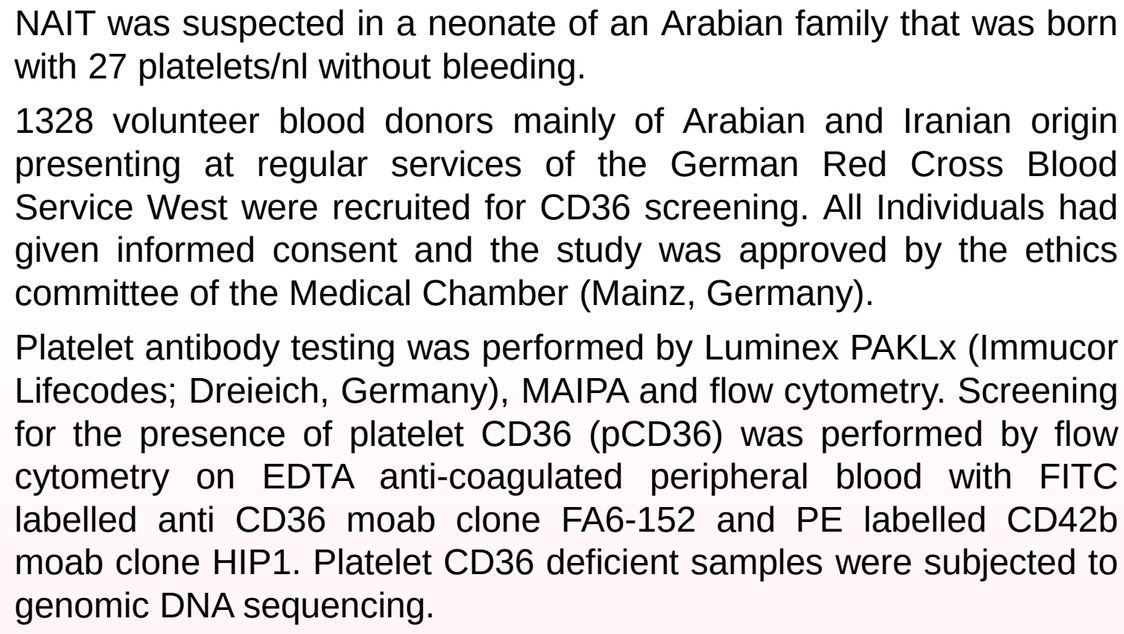

Fig. 1: Reactivity of the maternal serum and control (male AB serum) with random donor platelets (left, 1a). Double immunofluoresence staining showing lacking CD36 expression of the mother's monocytes (right, 1b). Flow Cytometer: Beckman Coulter CytoFlex.

Both, the mother's monocytes (fig. 1b) and platelets (fig. 2) completely lacked CD36 in flow cytometry in contrast to the platelets of father and infant (fig. 2). Thus, the mother showed a type 1 deficiency.

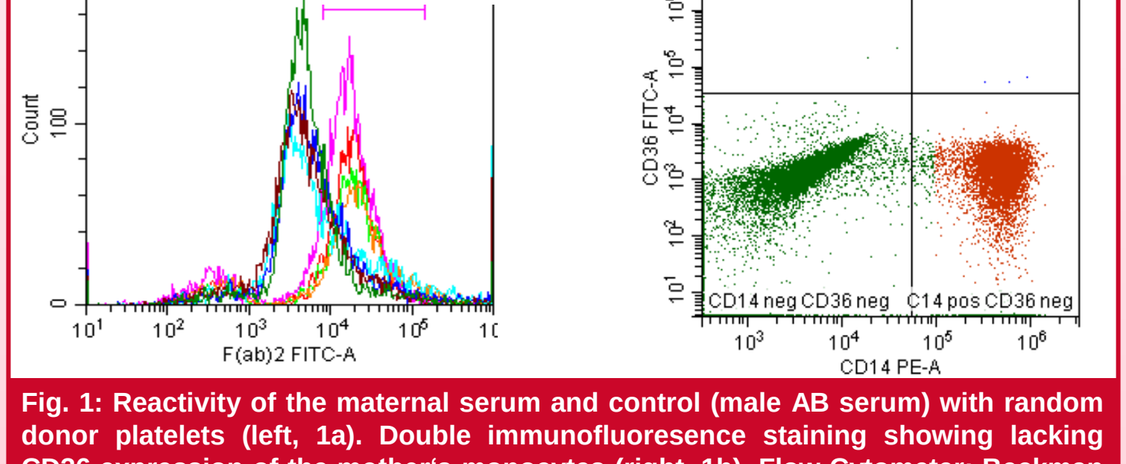

Fig. 2: Platelet CD36 (pCD36) expression of the mother, father and infant. Flow Cytometer: Beckman Coulter Cytoflex

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Because initially no CD36 compatible platelet apheresis concentrates (PC) were available, the infant was transfused with 2 random PC, that effectively could stabilize platelet counts (fig. 3).

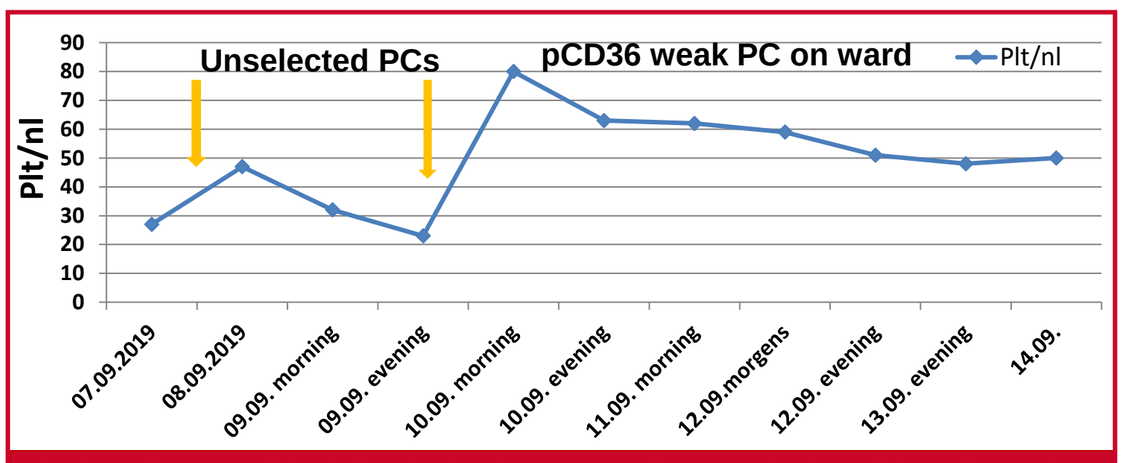

Fig. 3: Follow-up of the infant's platelet counts after transfusion of 2 random PC.

Compound heterozygosity for ***CD36*c.220C>T*** (**p.Q74X**) and ***CD36*c.1079T>G*** (**p.L360X**) was responsible for type 1 deficiency of the mother. She passed the ***CD36*c.1079T>G*** allele to her child.

Two Libanese brothers with a weak pCD36 expression donated apheresis platelets for the neonate. However, the products were not needed after the 2nd random transfusion. One donor homozygously carried a ***CD36*c.1076G>A*** **(p.G359E)** SNV while his brother showed compound heterozygosity for this SNV and a ***CD36*c.1202-1205delTATT → fs*** mutation.

A flow cytometric screening for pCD36 on 1328 volunteer blood donors from Arabian countries, Iran, Turkey, Sub-Saharan Africa and others identified **35 (2.6%) individuals as negative** and 19 (1,4%) with a weak expression (Table 1).

Table 1: Platelet CD36 expression in 1328 donors.

Region/ Country	Individuals [n]	Negative [n]	Negative [%]	Weak [n]	Weak [%]	Positive [n]
Syria	794	26	3.3	12	1.5	756
Arabian Peninsula	120	5	4.2	4	3.3	111
Northern Africa	32	0	0	0	0	32
Iran	147	3	2.0	1	0.7	143
Sub-Saharan Africa	39	1	2.6	1	2.6	37
Turkey	123	0	0	1	0.8	122
Others	73	0	0	0	0	73
Total	1328	35	2.6	19	1.4	1274

A total of 29 non-synonymous CD36 SNV and 12 mutations within flanking intron sequences was detected in the CD36 negative samples by DNA sequencing (table 2). Some of them already had been described before.

Table 2: SNV detected within 31 pCD36 negative samples with MFI values <1.0.
Flow cytometer: Beckman Coulter Epics XL.

No	SNV				
1	275C>G (T92R)	1156C>T (R386W)			
2	416A>G (I139V)				
3	446_449dupATCA→ fs				
4	447_450insTCAA→fs				
5	491>G (K164R)	503C>T (S168F)	539delG →fs	1133G>T (G378V)	1079T>G (L360X)
6	550G>A (Asp184Asn)	660_665delCATAAG → fs			
7	551A>T (D184V)				
8	638-639delAA → fs				
9	649G>A (G217R) homozygous				
10	649G>A (G217R)				
11	649G>A (G217R)	IVS5, 430-11t>g			
12	649G>A (G217R)	IVS8, 749-11t>g			
13	658_667del10 → fs				
14	660_664delCATAA → fs	1189G>T (E397X)			
15	660_664delCATAA →fs	1209ins16 → fs	IVS11, 1126-7c>a		
16	806C>A (S269Y)	IVS3, 121-6t>c			
17	871A>G (I291V)	IVS12, 1200-9_1208dup16 →fs			
18	923AG (D308G)	1189G>T (E397X)	3'UTR4_7 del agta		
19	957_958insA → fs	IVS12, 1200-12c>a			
20	975T>G (Y325X) homozygous				
21	1047_1051dupAAGT → fs	1345_1348delCTCA → fs			
22	1079T>G (L360X) homozygous				
23	1079T>G (L360X)	IVS3, 121-3t>G			
24	1079T>G (L360X)	1133G>T (G378V)			
25	1133G>T (G378V)	1215G>A (silent)			
26	1133G>T (G378V)				
27	1144C>T (Q382X)	IVS12, 1200-23_-8del16			
28	1156C>T (R386W)				
29	1156C>T (R386W)	IVS12, 1200-7a>g	3'UTRdel4		
30	1179_1183insCAGC → fs				
31	IVS12, 1200-53_-5inv49				

Conclusions

- Platelet CD36 deficiency is rather frequent in Arabian countries
- Type 1 deficiency enables CD36 isoimmunization and NAIT
- If no CD36 negative platelets should be available, the first choice is transfusion of ABO matched but further unselected platelets.
- Blood transfusion services should be aware of such cases.