Annals of Hematology (2020) 99:2731–2736
https://doi.org/10.1007/s00277-020-04104-4

ORIGINAL ARTICLE

Alloimmunization and autoimmunization in adult transfusion-dependent thalassemia patients: a report from a comprehensive center in Israel

Idit Pazgal[1,2] · Vered Yahalom[3] · Bruria Shalev[3] · Pia Raanani[1,2] · Pinhas Stark[1,2]

Received: 8 October 2019 / Accepted: 27 May 2020 / Published online: 2 June 2020

Abstract

Patients with beta thalassemia major (TM) are transfusion-dependent (TD) since early childhood and for life. Development of alloantibodies and autoantibodies against red blood cell (RBC) antigens is increasingly recognized as a significant transfusion hazard, especially among heavily transfused patients. The aim of this study is to assess RBC alloimmunization and autoimmunization rates in TD TM patients treated in our Comprehensive Center of Adult Thalassemia, Hemoglobinopathies and Rare Anemias. TD TM patients, regularly transfused every 2–3 weeks, were included in the study. Clinical and RBC transfusion records, including RBC antibodies, since diagnosis in early childhood, were retrieved from patients' files and from the blood bank database. Forty TD TM patients, > 18 years of age, were included in the study. Alloimmunization was demonstrated in 17 (42.5%) patients. Thirty-four alloantibodies were detected, with the most frequent being RH related (12 of 34, 35.3%) followed by those of the Kell system (8 of 34, 23.5%). Age at first transfusion was positively related to the probability of developing alloantibodies ($p = 0.02$). Splenectomy was found to be correlated with developing alloantibodies ($p = 0.016$). Logistic regression analysis of the lifelong probability of developing alloantibodies on the age at first transfusion and splenectomy demonstrates a strong positive relationship ($p = 0.002$). A substantially high rate of alloimmunization was found among adult TD TM patients. Early initiation of RBC transfusions, avoidance of splenectomy and extended Rh and K antigen matching, can reduce the incidence of alloimmunization in TD TM patients.

Keywords Thalassemia major · Alloimmunization · RBC transfusions

Introduction

Beta thalassemia is an inherited disorder resulting from mutation in the beta globin gene leading to impaired beta globin production, ineffective erythropoiesis, reduced red blood cell (RBC) survival, and chronic hemolytic anemia. Patients with severe form of the disease, i.e., beta thalassemia major (TM), are transfusion-dependent (TD) since early childhood and for life [1, 2]. Survival of TD TM patients has improved significantly over the past few decades as better treatment became available [3].

Nevertheless, development of anti-RBC alloantibodies and autoantibodies has been increasingly recognized as a major challenge of chronic transfusion therapy [4–6], as it significantly shortens in vivo survival of transfused RBC and limits availability of further safe transfusions [4, 7, 8].

Alloimmunization and autoimmunization rates observed in TD TM patients are highly variable and are in the range of 2.9–37.0% [9–15] and 0.0–40.0% [11, 16–18], respectively, whereas in the general population, the rates are in the range of 0.4–7.0% [5, 19–23], respectively. However, most reports are based mostly on TD TM pediatric patients, and less data is available on the rates in adult TD TM patients.

The aim of this study was to assess RBC alloimmunization and autoimmunization rates among the patients with TD TM

✉ Pinhas Stark
starkpinhas@gmail.com

[1] Comprehensive Center of Thalassemia, Hemoglobinopathies & Rare Anemias, Institute of Hematology, Beilinson Hospital, Rabin Medical Center, Petah Tikva, Israel

[2] Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

[3] Blood Services and Apheresis Institute, Rabin Medical Center, Petah Tikva, Israel

followed and treated in our Comprehensive Center of Adult Thalassemia, Hemoglobinopathies and Rare Anemias, in a 1300-bed major quaternary care and referral teaching medical center.

Materials and methods

Patients

The study cohort included TD TM patients, currently treated in our center. All patients were > 18 years of age, regularly transfused every 2–3 weeks. All patients received ABO and RhD compatible RBC transfusions collected by Magen David Adom (MDA, Israeli Red Cross organization) National Blood Services from voluntary donors according to national guidelines. Since the year 2004, all RBC transfusions have been leuko-reduced.

Patients' files and the blood bank database were screened for clinical data and transfusion records since the beginning of follow-up at early childhood. Data included date of birth, gender, ethnicity, ABO-D blood group, history of splenectomy, history of pregnancies, age at first transfusion, lifelong cumulative number of blood units transfused, and detected alloantibodies and autoantibodies, including specificities and age at first detection.

Laboratory investigations

Using standard blood bank methods [24], plasma was analyzed prior to each transfusion for detection of new antibodies to RBC antigens. The plasma was tested with commercially available screening cells using automated systems (IH1000 Bio-Rad, Hercules, California, USA, and AutoVue Ortho Clinical Diagnostics, Raritan, NJ, USA). A 3-cell screen was used for the antibody screening procedure, with low ionic strength solution (LISS)-IgG cards. If the antibody screen was positive, commercial panels (Bio-Rad, Hercules, CA, USA, and Ortho Clinical Diagnostics, Raritan, NJ, USA) including papain- and/or ficin-treated cells were performed to identify the antibodies as well as a direct anti globin test (DAT). Saline indirect anti-globulin test (SIAT) and polyethylene glycol test (PEG IAT) were used to further investigate and resolve the antibody reactivity. Adsorption methods were employed in patients presenting with a new autoantibody. In cases of a positive DAT, further investigation using specific reagents to detect IgG, or complement, was carried out. Eluates were prepared when IgG was detected on RBC and tested against commercial available panel cells. Cases that could not be resolved by the hospital blood bank laboratory, such as cases with multiple alloantibodies, those with a combination of alloantibodies and autoantibodies, or those that were recently transfused, were referred to MDA National Blood Group Reference Laboratory (MDA - NBGRL) for further investigation and confirmation. In case an alloantibody was identified, the information was documented in the blood bank records, and all subsequent RBC transfusions were negative for the corresponding antigen and cross-matched using LISS-IgG cards (Bio-Rad, Hercules, CA, USA). A notification letter with RBC antibodies details was sent to the patient.

Statistical analysis

Statistical analysis was performed using the SPSS Software, Version 26. Categorical variables were presented by (n, %) and analyzed using chi-square test, Fisher's exact test, or logistic regression with the Wald test. Continuous variables were presented by mean and SD, and were analyzed using nonparametric Wilcoxon two-sample test. p values < 0.05 were considered statistically significant.

Results

Forty patients were included in the study, 21 (52.5%) males and 19 (47.5%) females. There were 29 (72.5%) Muslim Arab patients and 11 (27.5%) Jewish. The median age was 31.0 years (range 20–47 years). The median age at first transfusion was 1.07 years (range 0.25–4.0 years). The median number of lifelong cumulative blood units transfused was 809 (range 439–1363). Thirty (75.0%) patients were splenectomized (SPX) and the median age at splenectomy was 10.0 (range 4.0–20.0). Patients' demographic and clinical characteristics are shown in Table 1.

Alloimmunization was found in 17 (42.5%) patients. Thirty-four alloantibodies were identified, with the most frequent being RH related (12 of 34, 35.3%) followed by those of the Kell system (8 of 34, 23.5%). Specificities of alloantibodies are shown in Table 2. Eleven of the 17 alloimmunized patients (64.7%) had multiple antibodies. Clinical characteristics of alloimmunized patients are shown in Table 3.

Autoimmunization was demonstrated in 2 (5.0%) patients; both had warm antibodies and had also alloantibodies.

Age at first transfusion was positively related to the probability of developing alloantibodies (chi-square test; $p = 0.02$), as shown by binary logistic regression analysis (odds ratio 2.476; 95% CI [1.040–5.895]; Wald test; $p = 0.04$). While starting transfusions at the age of 3 months led to a 26% lifelong probability of developing alloantibodies, starting transfusions at the age of 1 year led to a 41% lifelong probability of developing alloantibodies. Moreover, starting transfusions at the age of 3 years led to an 81% lifelong probability of developing alloantibodies.

Splenectomy was highly correlated with a lifelong development of alloantibodies, and the incidence of alloimmunization

Table 1 Demographic and clinical characteristics of 40 TD TM patients

Variable	Total $n = 40$ (100.0%)	Non-alloimmunized patients $n = 23$ (57.5%)	Immunized patients $n = 17$ (42.5%)
Age (years)			
Mean ± SD	31.8 ± 6.9	32.48 ± 6.83	30.94 ± 7.18
Median (range)	31.0 (20–47)	33.0 (20–45)	29.0 (22–47)
Gender			
Male	21 (52.5%)	11 (47.8%)	10 (58.8%)
Female	19 (47.5%)	12 (52.2%)	7 (41.2%)
Ethnicity			
Muslim Arab	29 (72.5%)	18 (78.3%)	11 (64.7%)
Jewish	11 (27.5%)	5 (21.7%)	6 (35.3%)
Blood group ABO			
A	17 (42.5%)	10 (43.5%)	7 (41.2%)
B	4 (10.0%)	2 (8.7%)	2 (11.8%)
O	19 (47.5%)	11 (47.8%)	8 (47.0%)
RhD			
D +	39 (97.5%)	23 (100%)	16 (94.1%)
D −	1 (2.5%)	0	1 (5.9%)
Age at first transfusion (years)			
Mean ± SD	0.71 ± 0.93	0.79 ± 0.50	1.46 ± 1.22
Median (range)	1.07 (0.25–4.0)	0.67 (0.25–2.50)	0.75 (0.25–4.0)
Total number of RBC transfused			
Mean ± SD	828.5 ± 239.3	858.0 ± 234.2	788.5 ± 247.3
Median (range)	809 (439–1363)	868 (439–1290)	730.0 (492–1363)
Splenectomy			
Yes	30 (75.0%)	18 (78.3%)	12 (70.6%)
No	10 (25.0%)	5 (21.7%)	5 (29.4%)
Age at splenectomy			
Mean ± SD	10.53 ± 4.15	9.22 ± 3.30	12.5 ± 4.64
Median (range)	10.0 (4.0–20.0)	9.0 (4.0–17.0)	11.0 (6.0–20.0%)

was significantly higher in splenectomized patients, compared with those with no splenectomy (chi-square test; $p = 0.016$).

Furthermore, logistic regression analysis of the lifelong probability of developing alloantibodies on the age at first transfusion and splenectomy demonstrated a strong positive relationship (Wald test; $p = 0.002$). The later transfusions started the higher the probability of developing alloantibodies (odds ratio 2.745; 95% CI [1.144–6.589]; Wald test; $p = 0.024$), and the performance of splenectomy greatly increased that same probability (odds ratio 6.957; 95% CI [1.497–32.325]; Wald test; $p = 0.013$). Starting transfusions at the age of 1 year and not having splenectomy at a later age led to a lifelong probability of 24% of developing alloantibodies, while having splenectomy at a later age led to a lifelong probability of 68% of developing alloantibodies.

In a series of logistic regressions, the incidence of alloimmunization found not to be influenced by patients' age, gender, ethnicity, duration of transfusion therapy, and cumulative number of blood units transfused ($p > 0.05$).

Discussion

This study is the first report from Israel showing a substantially high rate of alloimmunization among adult TD TM patients. Data analysis showed that alloimmunization rate was higher in patients who started transfusion later in life and had been splenectomized.

Anti-Rh and anti-Kell blood group system alloantibodies are the most frequently detected in the general population [19–21]. In our study, the most prevalent alloantibodies were against antigens of RH system (12 antibodies) and of Kell system (8 antibodies), similar to reports from other centers [4–7, 11, 16, 18, 23, 25].

Age at first transfusion was positively related to the probability of developing alloantibodies as starting transfusions at a later age were associated with higher alloimmunization rates. It has been previously shown that young age at the time of initial RBC exposure is associated with a significant lower likelihood of alloimmunization [16, 18, 23, 26]. The

Table 2 Specificity and prevalence of alloantibodies detected in 17 TD TM patients

Blood group system	Number (n = 34)	Frequency (% = 100)
Rh system	12	35.3%
Anti-E	8	23.5%
Anti-D	2	5.9%
Anti-C	1	2.9%
Anti-C^w	1	2.9%
Kell system	8	23.5%
Anti-K	6	17.6%
Anti-Kp^a	2	5.9%
Kidd system	1	2.9%
Anti-Jk^b	1	2.9%
Lewis system	1	2.9%
Anti-Le^a	1	2.9%
Lutheran system	1	2.9%
Anti-Lu^a	1	2.9%
Others	11	32.3%
Anti-Bg^a	5	14.7%
Low-frequency antigen*	3	8.8%
Others**	3	8.8%

* Low-frequency antigen: specificity not defined

** Others: anti-HI, anti-Mg, and high titer low avidity (HTLA)

immunologic mechanisms underlying these observations are not fully understood. It was suggested that patients at a very early age manifest immunological immaturity in many aspects, including humoral unresponsiveness to stimulations by carbohydrate antigens, hence resulting in immune tolerance to allogeneic RBC antigens and consequently reduced alloimmunization risk [23, 26, 27].

Splenectomy has been reported in several studies as a significant risk factor for alloimmunization [7, 23, 28]. The mechanism by which removal of the spleen increases alloantibody formation is not clear. It has been suggested that removal of the spleen led to non-filtering of antigens from the blood stream which results in higher alloantibodies production in splenectomized subjects [28]. It has also been hypothesized that post-splenectomy conformational changes in RBC membranes may enhance immunomodulation [6, 28]. In our study, splenectomy was highly correlated with alloantibodies development. Furthermore, logistic regression analysis of age at transfusion initiation and splenectomy found that splenectomy has a highly significant impact on the probability of developing alloantibodies.

All patients included in our study received RBC transfusions matched only for ABO and Rh(D). Matching RBC transfusions

Table 3 Clinical characteristics of 17 alloimmunized TD TM patients

Patient no.	Age (years)	Gender	Blood group ABO/Rh	Age at first transfusion (years)	Total number of RBC transfused	Age at splenectomy	Alloantibodies	Age at first alloantibody detection (years)
1	47	M	A+	0.5	1363	20	LF†, Bg^a	40
2	42	M	A+	0.5	1190	7	E, LF, Lu^a, Mg, HTLA *	28
3	39	M	A+	1.5	1068	12	HI	21
4	38	F	A+	3.5	999	10	K	24
5	37	M	B−	3.0	972	10	D, C, K	23
6	32	M	O+	2.5	807	19	E, Le^a	18
7	30	F	A+	2.0	747	ND	E, Bg^a	29
8	29	M	O+	0.75	730	17	E, K, Jk^b	11
9	29	M	A+	4.0	677	6	K	22
10	29	M	O+	0.5	736	10	E, K *	20
11	28	F	O+	0.33	707	17	C^w	26
12	26	F	O+	0.25	638	ND	E	12
13	25	F	O+	0.25	602	10	K, Kp^a, Bg^a	7
14	25	F	O+	2.0	573	ND	E	11
15	24	M	A+	0.5	565	ND	Kp^a, Bg^a	10
16	24	F	B+	2.0	539	12	Bg^a	6
17	22	M	O+	0.66	492	ND	D, E, LF **	8

† *LF* low-frequency antibodies, specificities not defined

* Patients no. 2 and no. 10 also had autoantibodies

** Patient no. 17, who is RhD+, had developed anti-D and a positive DCT. Consequently, he was transfused with RhD-RBC units. Anti-D disappeared within 4 months. The patient was switched back to transfusion with RhD+ RBC with no reemergence of anti-D, suggesting that the antibody observed was probably a transient auto-anti-D antibody

beyond ABO and Rh(D) antigens is currently considered as a preferred way to reduce alloimmunization in TD TM patients, although no standardized policies or consensuses have been widely implemented [5, 23, 29, 30]. Phenotypically matched RBC transfusions for Rh and Kell systems were reported to be associated with alloimmunization rates as low as 2.8–3.7% compared with rates as high as 22.6–33.0% observed in earlier studies [26, 28]. Moreover, it has been shown that RBC genotyping can improve matching of donor blood to patient, hence further reducing alloimmunization risk [27, 31]. However, in a recent study, despite showing that patients may benefit from receiving RBC transfusions based on genotyping, it was concluded that phenotypically matched RBC transfusions for ABO, Rh(D), and Kell systems seem to be already sufficient for RBC transfusions in thalassemia patients, preventing the development of alloantibodies [32].

Donor leukocytes within RBC units have been implicated in stimulating alloimmunization, and consequently, the use of leuko-reduced blood has been widely implemented in effort to reduce alloimmunization. Currently, most blood transfused in the USA, in Canada, and in many other developed countries is pre-storage leuko-reduced [23, 28, 33]. In our center, all adult patients were exposed to non-leuko-reduced blood, as universal leuko-reduction has been implemented in Israel since 2004. This might have contributed to our high rates of red cell alloimmunization.

The incidence of alloimmuniuzation was not influenced by patients' age, gender, ethnicity, duration of transfusion therapy, and cumulative number of blood units transfused, in contrast to what is described in the literature [21, 23, 34], probably due to the limitation of this retrospective observational study of a relatively small cohort size.

In conclusion, we have found a substantially high rate of alloimmunization in adult TD TM patients, which might be attributed to donor/recipient heterogeneity and to multi-transfusions over many years without RBC antigen matching beyond ABO and RhD, and without leuko-reduction. Alloimmunization rate was higher in patients who started transfusion later in life and had been splenectomized. Early diagnosis of TD TM as well as early initiation of blood transfusions, avoidance of splenectomy, and implementation of Cc, Ee, and K antigen matching may reduce the incidence of alloimmunization in TD thalassemia patients.

Acknowledgments The authors would like to thank Prof. Amit Pazgal, from Jesse H. Jones Graduate School of Business, Rice University, Houston, TX 77005, USA, for his comprehensive statistical analysis.

Compliance with ethical standards

Ethical approval The study was approved by the local Institutional Review Board. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Olivieri NF (1999) The beta-thalassemias. N Engl J Med 341:99–109. https://doi.org/10.1056/NEJM199907083410207
2. Rund D, Rachmilewitz E (2005) Beta-thalassemia. N Engl J Med 353:1135–1146. https://doi.org/10.1056/NEJMra050436
3. Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, Ghilardi R, Origa R, Piga A, Romeo MA, Zhao H, Cnaan A (2005) Survival and complications in thalassemia. Ann N Y Acad Sci 1054:40–47. https://doi.org/10.1196/annals.1345.006
4. Matteocci A, Pierelli L (2014) Red blood cell alloimmunization in sickle cell disease and in thalassaemia: current status, future perspectives and potential role of molecular typing. Vox Sang 106:197–208. https://doi.org/10.1111/vox.12086
5. Chou ST, Liem RI, Thompson AA (2012) Challenges of alloimmunization in patients with haemoglobinopathies. Br J Haematol 159:394–404. https://doi.org/10.1111/bjh.12061
6. Thompson AA, Cunningham MJ, Singer ST, Neufeld EJ, Vichinsky E, Yamashita R, Giardina P, Kim HY, Trachtenberg F, Kwiatkowski JL, Thalassemia Clinical Research Network Investigators (2011) Red cell alloimmunization in a diverse population of transfused patients with thalassaemia. Br J Haematol 153:121–128. https://doi.org/10.1111/j.1365-2141.2011.08576.x
7. Hendrickson JE, Tormey CA (2016) Red blood cell antibodies in hematology/oncology patients: interpretation of immunohematologic tests and clinical significance of detected antibodies. Hematol Oncol Clin N Am 30:635–651. https://doi.org/10.1016/j.hoc.2016.01.006
8. El Danasoury AS, Eissa DG, Abdo RM, Elalfy MS (2012) Red blood cell alloimmunization in transfusion-dependent Egyptian patients with thalassemia in a limited donor exposure program. Transfusion 52:43–47. https://doi.org/10.1111/j.1537-2995.2011.03234.x
9. Wang LY, Liang DC, Liu HC, Chang FC, Wang CL, Chan YS, Lin M (2006) Alloimmunization among patients with transfusion-dependent thalassemia in Taiwan. Transfus Med 16:200–203. https://doi.org/10.1111/j.1365-3148.2006.00656.x
10. Sadeghian MH, Keramati MR, Badiei Z, Ravarian M, Ayatollahi H, Rafatpanah H, Daluei MK (2009) Alloimmunization among transfusion-dependent thalassemia patients. Asian J Transfus Sci 3:95–98. https://doi.org/10.4103/0973-6247.53884
11. Pahuja S, Pujani M, Gupta SK, Chandra J, Jain M (2010) Alloimmunization and red cell autoimmunization in multitransfused thalassemics of Indian origin. Hematology 15:174–177. https://doi.org/10.1179/102453309X12583347114013
12. Sirchia G, Zanella A, Parravicini A, Morelati F, Rebulla P, Masera G (1985) Red cell alloantibodies in thalassemia major: results of an Italian cooperative study. Transfusion 25:110–112. https://doi.org/10.1046/j.1537-2995.1985.25285169198.x
13. Ameen R, Al-Shemmari S, Al-Humood S, Chowdhury RI, Al-Eyaadi O, Al-Bashir A (2003) RBC alloimmunization and autoimmunization among transfusion-dependent Arab thalassemia patients. Transfusion 43:1604–1610. https://doi.org/10.1046/j.1537-2995.2003.00549.x
14. Zahran AM, Elsayh KI, Saad K, Embaby M, Ali AM (2016) Regulatory B cells ($CD19^{+}CD38^{hi}CD24^{hi}$) in alloimmunized and non-alloimmunized children with beta thalassemia major. Blood Cells Mol Dis 57:91–96. https://doi.org/10.1016/j.bcmd.2016.01.005

15. Karimi M, Nikrooz P, Kashef S, Jamalian N, Davatolhagh Z (2007) RBC alloimmunization in blood transfusion-dependent beta-thalassemia patients in southern Iran. Int J Lab Hematol 29:321–326. https://doi.org/10.1111/j.1365-2257.2006.00856.x
16. Al-Mousawi MMN, Al-Allawi NAS, Alnaqshabandi R (2015) Predictors of red cell alloimmunization in Kurdish multi transfused patients with hemoglobinopathies in Iraq. Hemoglobin 39:423–426. https://doi.org/10.3109/03630269.2015.1077460
17. Guirat-Dhouib N, Mezri M, Hmida H, Mellouli F, Kaabi H, Ouderni M, Zouari B, Hmida S, Bejaoui M (2011) High frequency of autoimmunization among transfusion-dependent Tunisian thalassaemia patients. Transfus Apher Sci 45:199–202. https://doi.org/10.1016/j.transci.2011.08.003
18. Dhawan HK, Kumawat V, Marwaha N, Sharma RR, Sachdev S, Bansal D, Marwaha RK, Arora S (2014) Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients: study on 319 patients. Asian J Transfus Sci 8:84–88. https://doi.org/10.4103/0973-6247.137438
19. Hoeltge GA, Domen RE, Rybicki LA, Schaffer PA (1995) Multiple red cell transfusions and alloimmunization. Experience with 6996 antibodies detected in a total of 159,262 patients from 1985 to 1993. Arch Pathol Lab Med 119:42–45
20. Tormey CA, Fisk J, Stack G (2008) Red blood cell alloantibody frequency, specificity, and properties in a population of male military veterans. Transfusion 48:2069–2076. https://doi.org/10.1111/j.1537-2995.2008.01815.x
21. Karafin MS, Westlake M, Hauser RG, Tormey CA, Norris PJ, Roubinian NH, Wu Y, Triulzi DJ, Kleinman S, Hendrickson JE, NHLBI Recipient Epidemiology and Donor Evaluation Study-III (REDS-III) (2018) Risk factors for red blood cell alloimmunization in the Recipient Epidemiology and Donor Evaluation Study (REDS-III) database. Br J Haematol 181:672–681. https://doi.org/10.1111/bjh.15182
22. Wheeler CA, Calhoun L, Blackall DP (2004) Warm reactive autoantibodies: clinical and serologic correlations. Am J Clin Pathol 22:680–685. https://doi.org/10.1309/CJAW-6N8J-6H0H-R2WM
23. Vichinsky E, Neumayr L, Trimble S, Giardina PJ, Cohen AR, Coates T, Boudreaux J, Neufeld EJ, Kenney K, Grant A, Thompson AA, Thalassemia Investigators CDC (2014) Transfusion complications in thalassemia patients: a report from the Centers for Disease Control and Prevention. Transfusion 54:972–981. https://doi.org/10.1111/trf.12348
24. Fung MK, Eder A, Spitalnik SL, Westhoff CM (2017) AABB technical manual, 19th edn. Bethesda, MD: American Association of Blood Banks. ISBN 978-1-56395-947-9
25. Gupta R, Singh DK, Singh B, Rusia U (2011) Alloimmunization to red cells in thalassemics: emerging problem and future strategies. Transfus Apher Sci 45:167–170. https://doi.org/10.1016/j.transci.2011.07.014
26. Spanos T, Karageorga M, Ladis V, Peristeri J, Hatziliami A, Kattamis C (1990) Red cell alloantibodies in patients with thalassemia. Vox Sang 58:50–55. https://doi.org/10.1111/j.1423-0410.1990.tb02055.x
27. Hendrickson JE, Tormey CA, Shaz BH (2014) Red blood cell alloimmunization mitigation strategies. Transfus Med Rev 28:137–144. https://doi.org/10.1016/j.tmrv.2014.04.008
28. Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP (2000) Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. Blood 96:3369–3373
29. Compernolle V, Chou ST, Tanael S, Savage W, Howard J, Josephson CD, Odame I, Hogan C, Denomme G, Shehata N, International Collaboration for Transfusion Medicine Guidelines (2018) Red blood cell specifications for patients with hemoglobinopathies: a systematic review and guideline. Transfusion 58:1555–1556. https://doi.org/10.1111/trf.14611
30. Ambruso DR (2014) Transfusion complications in thalassemias (ed.). Transfusion 54:957–959. https://doi.org/10.1111/trf.12576
31. Avent ND (2009) Large-scale blood group genotyping: clinical implications. Br J Haematol 144:3–13. https://doi.org/10.1111/j.1365-2141.2008.07285.x
32. Belsito A, Costa D, Signoriello S, Fiorito C, Tartaglione I, Casale M, Perrotta S, Magnussen K, Napoli C (2019) Clinical outcome of transfusions with extended red blood cell matching in beta-thalassemia patients: a single-center experience. Transfus Apher Sci 58:65–71. https://doi.org/10.1016/j.transci.2018.11.006
33. Blumberg N, Heal JM, Gettings KF (2003) WBC reduction of RBC transfusions is associated with a decreased incidence of RBC alloimmunization. Transfusion 43:945–952. https://doi.org/10.1046/j.1537-2995.2003.00443.x
34. Elhence P, Solanki A, Verma A (2014) Red blood cell antibodies in thalassemia patients in northern India: risk factors and literature review. Indian J Hematol Blood Transfus 30:301–308. https://doi.org/10.1007/s12288-013-0311-y