

Evanescence and persistence of red blood cell antibodies over time: a single-center experience

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Alloantibodies may develop after exposure to foreign red blood cell (RBC) antigens. Evanescence occurs when an antibody falls below the sensitivity threshold of methods used in pre-transfusion testing. An alloantibody that has evanesced may go undetected, resulting in possible delayed hemolytic transfusion reactions, which lead to increased morbidity and mortality. A survey was conducted to analyze evanescence of alloantibodies over time. A total of 544 patients with 656 alloantibodies were evaluated. Median follow-up was 294 days (range 3–3852 days). Analysis showed that patient age at detection of alloantibody ($p = 0.037$), sex ($p < 0.001$), results of initial RBC antibody screen ($p < 0.001$), RBC transfusion ($p < 0.001$), length of follow-up period ($p < 0.001$), and alloantibody specificity ($p = 0.004$) significantly influenced the time of evanescence. Evanescence rate was the highest for anti-Jk^a, anti-C, and anti-M and the lowest for anti-Fy^a and anti-D specificities. Evanescence of alloantibodies represents a significant problem in routine pre-transfusion testing. Beyond improving testing by implementing more sensitive methods, there is a place for preventive usage of extended antigen-matched RBC units or the application of post-transfusion protocols. Sharing of antibody information across centers can also improve transfusion safety in these centers. *Immunohematology* 2025;41:124–131. DOI: 10.2478/immunohematology-2025-018.

Key Words: alloantibody evanescence, alloantibody persistence, red blood cell antibodies

Red blood cell (RBC) alloantibodies develop after exposure to foreign RBC antigens during blood transfusion, pregnancy, and/or organ, cell, and tissue transplantation. Several factors may affect the immune response to RBC antigens. The immunogenicity of blood group antigens is the most prominent, but other factors also play an important role, including the unique set of genes where specific human leukocyte antigen alleles associated with alloimmunization are located,^{1,2} the specific disease state of each patient, the genetics and physiologic state of the donor, and the preparation and storage of the RBC unit.^{3–6} Over the past 10–15 years, numerous mouse models have been developed that allow

the investigation of topics that are simply not feasible to study in humans because studies of factors influencing RBC alloimmunization (other than RBC immunogenicity and the clinical significance of antibodies in humans) are limited by a large number of independent variables.⁷

Antibody presence in the patient's plasma is detected by the RBC antibody detection test used for pre-transfusion testing. Detection of antibodies is determined by the test sensitivity and frequency of pre-transfusion testing. Depending on the study population and design, rates of alloimmunization range from approximately 2 to 6 percent in general transfused patients and are higher in transfusion-dependent patients (e.g., up to 44% in sickle cell disease, from 5 to 45% in thalassemia, and from 15 to 59% in myelodysplastic syndrome).^{8–10}

Complete evanescence of an antibody occurs when the antibody is no longer detectable by standard methods. If there is no history of the antibody, and it goes undetected in pre-transfusion testing, there is risk of a delayed hemolytic transfusion reaction if the patient is transfused with a unit positive for the corresponding antigen.

It is estimated that approximately two-thirds of all antibodies will eventually fade away.¹¹ The mechanism of RBC antibody evanescence is not well understood. Factors that may be involved in the evanescence of antibodies depend on patient characteristics, such as the immune status and underlying disease, therapy, medications, transfusion, pregnancy status, and antibody specificity. The length of the follow-up period strongly influences evanescence of antibodies; the longer the follow-up period, the higher the reported evanescence rate. Retrospective reports have shown that one-quarter of induced antibodies can disappear from detection within 1 month of their initial discovery, with one-half of the antibodies undetectable at 6 months and two-thirds undetectable at 5 or more years after initial detection.¹² In this study, factors

influencing the evanescence of RBC alloantibodies in hospitalized patients during the 12-year period were evaluated.

Materials and Methods

Patients

A retrospective analysis of records was conducted for patients with alloantibodies identified between January 2011 and December 2019, with a follow-up period until December 2022. This study was approved by the institutional ethical committee (class 8.1-22/137-2, number 02/013 AG). Only complete records of alloimmunized patients, who underwent at least one follow-up RBC antibody screening after the initial screening, were studied. Patients with only cold-reacting alloantibodies, warm or cold autoantibodies, and anti-D due to anti-D prophylaxis were excluded from the analysis. Information on anti-D prophylaxis was available for all patients with passively acquired anti-D. Cases with anti-C and anti-D were not always evaluated for anti-G. In men and women of non-reproductive age, anti-G was excluded based on weaker reactions for anti-C than anti-D or whether anti-C and anti-D were developed or had disappeared separately. In patients younger than 1 year of age, passively acquired maternal alloantibodies were excluded. The study included RBC alloantibodies directed against antigens found in the RH, KEL, JK, FY, MNS, LE, and LU blood group systems. Antibodies directed toward low-prevalence antigens such as C^w, Lu^a, and Kp^a were included in the study only if multiple antibody specificities were present.

Data Collection

Data were collected from two databases: the transfusion laboratory information system for results of RBC antibody detection tests and transfusion history, and the hospital information system for clinical data. Analyzed data were (1) patient age at detection of alloantibody, (2) sex, (3) results of initial RBC antibody screen, (4) RBC transfusion, (5) length of follow-up period, (6) antibody specificity, (7) presence of multiple alloantibodies, (8) evanescence rate, and (9) duration of detectability. Information was not available for previous pregnancies or antibodies detected at other facilities. In cases of multiple RBC alloantibodies, antibody identification records for each antibody were reviewed separately.

Serologic Testing

From January 2011 to July 2019, patients' blood samples were screened for alloantibodies using a two-cell set of reagent

RBCs (ID-DiaCell I-II; Bio-Rad Laboratories, Puchheim, Germany) for antibody detection for most of the study period and afterwards using a three-cell set (Surgiscreen; QuidelOrtho, Bridgend, UK). For RBC antibody identification, 11-cell reagent RBC panels, both untreated and enzyme-treated, were used (ID-DiaPanel, ID-DiaPanel-P; Bio-Rad Laboratories) (Resolve Panel C, Resolve Panel C ficin-treated; QuidelOrtho). For complex cases, additional identification panels were used.

After the first positive RBC antibody detection test, an antibody investigation was performed to identify the specificity of the antibody(ies). In subsequent pre-transfusion testing, a new antibody identification panel was tested if the RBC antibody detection test was positive and >72 hours had passed from the previous identification panel.

If a subsequent RBC antibody detection test or identification panel was found negative, the antibody was regarded as evanescent and no longer present in the bloodstream. The antibody was regarded as persistent if it was present on the last RBC antibody detection test or identification panel.

Statistical Analysis

The study was designed as a survival analysis with the primary outcome as the time to antibody evanescence. Evanescent antibodies were compared considering patient age at detection of alloantibody, sex, results of initial RBC antibody screen, RBC transfusion, length of follow-up period, antibody specificity, and presence of multiple alloantibodies. The Cox proportional hazard regression analysis was performed to establish the impact of factors on antibody evanescence in time. The Kaplan-Meier estimator was used to visually demonstrate the rate at which the antibodies became evanescent. A statistical software package (SPSS version 26.0; IBM) was used for the statistical analyses. It was assumed that there was a statistical difference if the probability level was <0.05.

Results

Medical records of 1967 patients with RBC alloantibodies identified over 9 years were evaluated, and 544 met the inclusion criteria. The study included 355 (65.2%) female patients and 189 (34.8%) male patients, with a median age of 64 years (range 0–93 years) at the time of antibody detection. In this group, 455 patients had one alloantibody and 89 had multiple antibodies. Of the 656 alloantibodies evaluated, 277 were formed after the initial RBC antibody screen was negative. A total of 420 patients received RBC transfusions

during the study timeframe. The most frequent antibody specificities detected were anti-E ($n = 152$, 23.2%), anti-K ($n = 132$, 20.1%), and anti-D ($n = 126$, 19.2%). The median follow-up period for all patients was 294 days (range 3–3852 days), and over the follow-up period, 218 (33.2%) alloantibodies became undetectable. Antibodies detected within the 3-day timeframe used for reporting evanescent antibodies were likely newly identified antibodies, possibly IgM in nature, which disappeared in 3 days. Patients were not followed up a second time, so we were unable to determine whether these antibodies may have developed an IgG component, which would possibly be persistent.

Further subsets of data were analyzed. We noted that 277 antibodies (42.2%) were formed after a negative initial RBC antibody screen. Of the most frequent alloantibody specificities (occurring in more than 10 patients), the highest evanescence rates occurred with anti-Jk^a (27 of 44, 61.4%), anti-M (29 of 61, 47.5%), and anti-C (26 of 59, 44.1%) (Table 1). Analysis of specificities occurring in less than 10 patients may not provide statistically supported accurate information (anti-C^w, anti-Kp^a, anti-e, anti-G, anti-Jk^b, anti-k, and anti-Lu^a). The number of evanesced antibodies for other variables were included: male

versus female (101 vs. 117), younger versus older than 50 years (43 vs. 175), initial RBC antibody screen positive versus negative (138 vs. 80), patients transfused with RBC units versus patients not transfused (33 vs. 185), and patients with single versus patients with multiple antibodies (149 vs. 69).

The univariate Cox proportional hazard regression function showed that patient age at detection of alloantibody ($p = 0.037$), sex ($p < 0.001$), results of initial RBC antibody screen ($p < 0.001$), RBC transfusion ($p < 0.001$), length of follow-up period ($p < 0.001$), and antibody specificity ($p = 0.004$) significantly influenced the time of evanescence, while the presence of multiple antibodies had no influence (Table 2). Multivariate analysis showed that sex, results of the initial RBC antibody screen, and antibody specificity remained associated with antibody evanescence (Table 3).

A Kaplan-Meier estimate of the cumulative probability function for antibodies detected during the study illustrates the relationship between the patient age at detection of alloantibody ($p = 0.036$), sex ($p < 0.001$), results of the initial RBC antibody screen ($p < 0.001$), RBC transfusion ($p < 0.001$), antibody specificity ($p < 0.001$), and the length of time the antibody was detectable (Fig. 1). Analyses showed

Table 1. Antibody specificity, evanescence, and persistence over the follow-up period

Antibody specificity	Total alloantibodies ($N = 656$)		Evanescent alloantibodies ($n = 218$)		Persistent alloantibodies ($n = 438$)		Total follow-up (days)	
	n	%	n	%*	n	%*	Median	Range
Anti-E	152	23.2	56	36.8	96	63.2	246.5	3–2788
Anti-K	132	20.1	42	31.8	90	68.2	276.5	5–3849
Anti-D	126	19.2	12	9.5	114	90.5	426	4–3847
Anti-M	61	9.3	29	47.5	32	52.5	297	3–3842
Anti-C	59	8.8	26	44.1	33	55.9	459	3–3847
Anti-Jk ^a	44	6.7	27	61.4	17	38.6	151	3–2788
Anti-c	28	4.3	9	32.1	19	67.9	179	3–3971
Anti-Fy ^a	19	2.8	0	0	19	100.0	405	5–2559
Anti-S	11	1.7	4	36.4	7	63.6	1743	7–2458
Anti-C ^w	8	1.2	4	50.0	4	50.0	1079	15–2546
Anti-Kp ^a	5	0.8	3	60.0	2	40.0	274	7–1610
Anti-e	3	0.5	1	33.3	2	66.7	254	117–2337
Anti-Fy ^b	3	0.5	2	66.7	1	33.3	113	5–435
Anti-G	2	0.3	0	0	2	100.0	1827	59–3595
Anti-Jk ^b	1	0.2	1	100.0	0	0	754	NA
Anti-k	1	0.2	0	0	1	100.0	93	NA
Anti-Lu ^a	1	0.2	1	100.0	0	0	710	NA

*Percentage of this alloantibody.

NA = not applicable.

Table 2. Univariate Cox proportional hazard regression of antibody evanescence

Variable	Hazard ratio	95% CI	p
Patient age at detection of alloantibody	1.427	1.021–1.995	0.037
Sex	0.606	0.464–0.791	<0.001
Results of initial RBC antibody screen	0.277	0.208–0.367	<0.001
RBC transfusion	2.125	1.464–3.085	<0.001
Presence of multiple antibodies	0.828	0.622–1.102	NS
Length of follow-up period	0.166	0.114–0.242	<0.001
Antibody specificity	1.070	1.022–1.121	0.004
Anti-C	4.387	2.213–8.698	<0.001
Anti-E	5.352	2.866–9.995	<0.001
Anti-c	4.518	1.902–10.731	0.001
Anti-K	3.827	2.014–7.272	<0.001
Anti-Jk ^a	10.334	5.222–10.453	<0.001
Anti-Fy ^a	0.000	0.000–1.041E+132	NS
Anti-S	2.912	0.938–9.042	NS
Anti-M	5.950	3.036–11.664	<0.001
Anti-D	1 (Reference)		

CI = confidence interval; RBC = red blood cell; NS = not significant.

that antibodies in patients older than 50 years, male patients, patients with negative initial results of RBC antibody screen, or patients transfused with RBC units had higher antibody evanescence rates. The evanescence rate of antibodies was the highest for anti-Jk^a, anti-C, and anti-M specificities and the lowest for anti-Fy^a and anti-D specificities.

Discussion

This study investigated factors that influence evanescence of RBC alloantibodies. Because antibodies may become undetectable over time, patients are at risk of a delayed hemolytic transfusion reaction due to transfusion of an RBC unit that is positive for the corresponding antigen. Risk is further increased when immunohematology testing results are not shared between hospitals nor are antibodies reported to the national antibody register. We found an evanescence rate of 33 percent in all hospitalized patients for a median follow-up period of 9.8 months (range 1–128 months). The evanescence rate reported in the literature for patients of both sexes ranged from 26 to 37 percent for a similar follow-up period.^{13,14}

Table 3. Multivariate Cox proportional hazard regression of antibody evanescence

Variable	Hazard ratio	95% CI	p
Patient age at detection of alloantibody	1.328	0.929–1.899	NS
Sex	0.660	0.4970.875	0.004
Results of initial RBC antibody screen	0.355	0.261–0.485	<0.001
RBC transfusion	1.340	0.901–1.994	NS
Presence of multiple antibodies	0.887	0.646–1.219	NS
Length of follow-up period	0.160	0.160–0.242	<0.001
Antibody specificity	1.054	1.002–1.109	0.044
Anti-C	4.190	2.075–8.460	<0.001
Anti-E	3.334	1.731–6.421	<0.001
Anti-c	3.093	1.268–7.545	0.013
Anti-K	2.873	1.488–5.546	0.002
Anti-Jk ^a	5.859	2.892–11.870	<0.001
Anti-Fy ^a	0.000	0.000–5.570E+121	NS
Anti-S	2.037	0.640–6.483	NS
Anti-M	5.044	2.496–10.190	<0.001
Anti-D	1 (Reference)		

CI = confidence interval; RBC = red blood cell; NS = not significant.

In this study, antibody evanescence was significantly influenced by the patient's age and sex. Patients older than 50 years and male patients had more evanescent antibodies than younger patients and female patients. The significance remained also in the multivariate analysis for sex but was lost for age. It is possible that the weaker immunologic status of older patients and the comorbidities that usually go along with age added to the faster evanescence of antibodies. As for sex, the longer persistence of alloantibodies in female individuals could be attributed to prolonged exposure to pregnancy-induced microchimerism.¹⁵ Verduin et al.¹⁶ analyzed the persistence of RBC antibodies in women after pregnancies complicated by hemolytic disease of the fetus and newborn (HDFN) treated with intrauterine transfusions. After a median follow-up of 8.7 years, all 260 antibodies primarily responsible for HDFN had persisted, while additional antibodies directed against the antigens of children persisted in 70.6 percent and in 30.3 percent of cases if they were not child-specific ($p < 0.001$). The difference between fetal and non-fetal immunogens suggests maintenance of antigenic stimulation possibly by long-term feto-maternal chimerism. In two other studies that

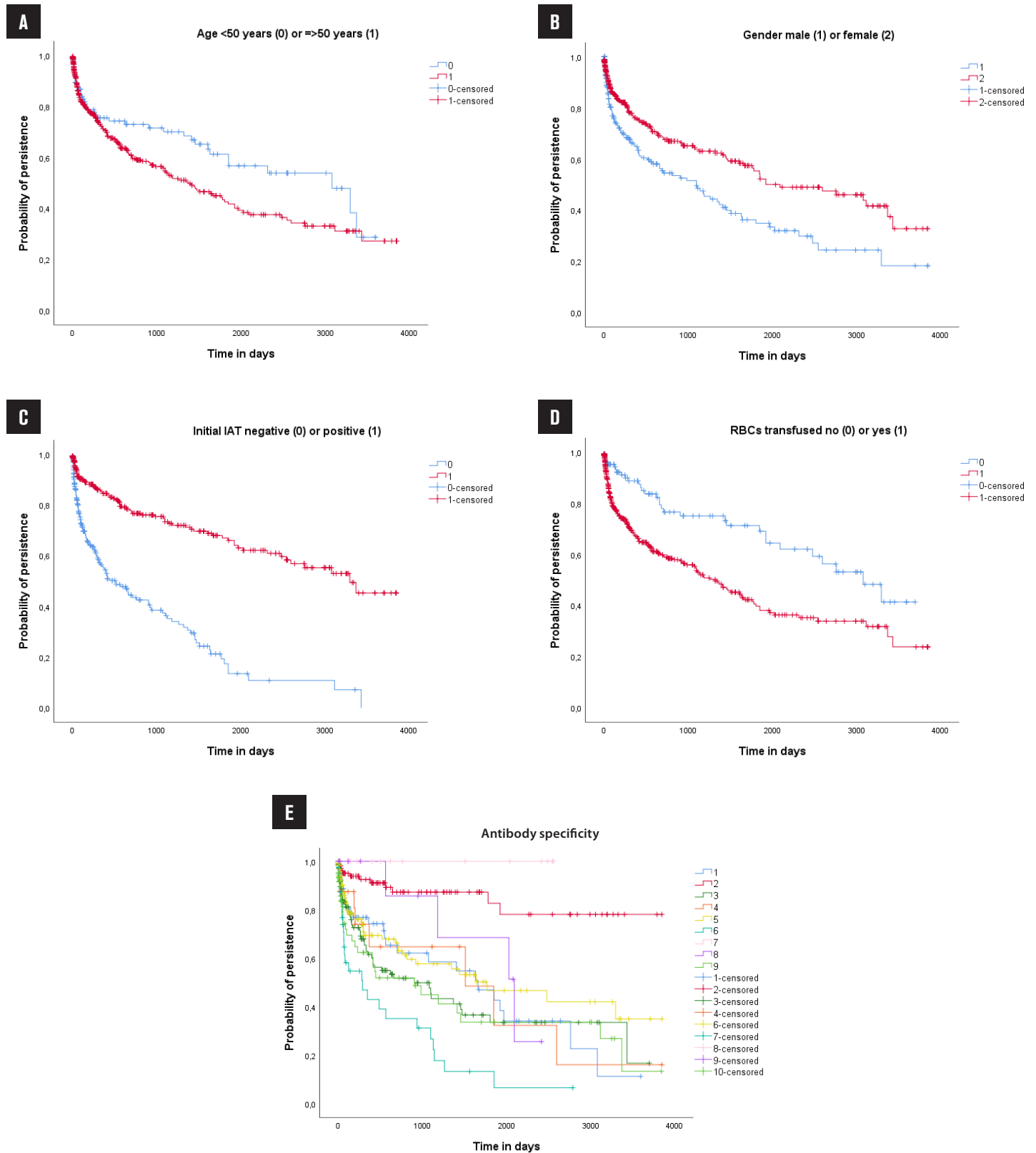


Fig. 1 Kaplan-Maier curves of patient age at detection of alloantibody (A), sex (B), results of initial red blood cell (RBC) antibody screening (C), RBC transfusion (D), and antibody specificity (E). Censored cases are patients who experienced antibody evanescence during the study period.

analyzed mixed-sex patient populations, age and sex were not statistically significant covariates for antibody persistence.^{13,14} Both studies showed that the testing method/manner influenced the detection of persisting antibodies. In the study by Schonewille et al.,¹³ RBC antibodies detected by the tube albumin–indirect antiglobulin test (IAT) method persisted longer than those found by the column low-ionic-strength saline–IAT method, while in the study by Reverberi,¹⁴ more frequent testing, lower maximum reaction scores, and a longer follow-up (median 2 vs. 1, 2 vs. 3, and 409 vs. 236 days for non-persistent vs. persistent antibodies) were associated with non-persistent antibodies. In this study, patients with a positive initial RBC antibody screen had significantly lower evanescence of antibodies compared with patients with negative initial results, which is in concordance with the results of other studies.^{12,17} In a study on male veterans, Tormey and Stack¹² showed that the evanescence rate of hospital-acquired antibodies was significantly higher than that of preexisting antibodies. The highest evanescent rate of 64 percent was observed for hospital-acquired antibodies tested 5 or more years after initial development, which is also dependent on the duration of follow-up testing. This rate in male patients was the highest reported evanescence in comparison with previously reported rates in mixed-sex populations. In another study on donors, which represents a healthy population, antibodies detected at the first donation had a higher rate of persistence than those detected during the study.¹⁷ Two studies showed this decrease was not a first-order decay process.^{12,17} The explanation might be the connection with younger age at the time of alloimmunization and durable immune response or simply that persistent antibodies were randomly included in the study.

In our study, RBC transfusion significantly influenced the antibody evanescence. Patients who were not transfused had a higher persistence of antibodies in comparison with patients who received blood transfusion. However, significance did not remain in the multivariate analysis. Hauser et al.¹⁷ reported that the donors with a history of past transfusions had antibodies that were highly persistent, which could not be confirmed with our study. It is probable that immunomodulatory effects of blood transfusion could also contribute to a higher evanescence rate in transfused populations,¹⁸ but this discrepancy should be further investigated in larger studies.

A study by Noiret et al.¹⁹ analyzed the persistence in multiply alloimmunized patients with simultaneously identified antibodies (antibodies discovered on the same antibody screen) and sequentially identified antibodies (different

antibodies discovered on different sequential antibody screens). In their study, patients with simultaneously identified antibodies had significantly more persistent antibodies than patients with sequentially identified antibodies (mean 9.2 vs. 4.9 months; $p < 0.001$).¹⁹ Hauser et al.¹⁷ demonstrated a greater persistence rate in multiply alloimmunized donors with antibodies detected concurrently than in donors with a single antibody. In our study, patients with multiple antibodies did not show a difference in the evanescence of antibodies compared with patients with single antibodies.

Analysis showed that the detection of antibody was significantly influenced by the length of the follow-up period; the evanescence rate was higher for the longer follow-up period (≥ 6 months compared with < 6 months). In the study by Reverberi,¹⁴ non-persistent antibodies were followed up for a longer time (median 409 vs. 236 days, $p = 0.012$). Likewise, Tormey and Stack¹² reported that the evanescence rate of hospital-acquired alloantibodies was the highest with the longest follow-up testing: antibodies with > 5 years of follow-up testing had a disappearance rate of 64 percent.¹² The difference was significant for antibodies with ≤ 1 year of follow-up testing compared with antibodies with > 1 year of testing (36 vs. 58%, $p < 0.01$).

Antibody detection is associated with antibody specificity. We found anti-Fy^a and anti-D were the least evanescent antibodies, and anti-Jk^a, anti-C, and anti-M were the most evanescent antibodies among the most frequent specificities identified. This finding agrees for both anti-D^{12–14,17} and anti-Fy^a.^{12,17} Also, this finding agrees for anti-Jk^a^{12–14,17} and anti-M^{12,17} but not for anti-C. In the study by Schonewille et al.,⁵ anti-Jk^a and anti-Jk^b were predominantly found in patients tested within 3 months, whereas anti-K and anti-Fy^a were the most encountered antibodies at > 5 years after transfusion. In the study by Tormey and Stack,¹² persistence of anti-C did not differ from anti-D. Anti-C is often present together with anti-D, but the persistence might be different if each antibody is followed separately, which was the case in our study.

A study by Stack and Tormey¹¹ identified the detection rate of alloantibodies to be 31.6 percent. This finding indicated that previously reported alloimmunization rates in general hospitalized populations have been significantly underestimated. In the study by Dinh et al.,²⁰ patients in whom antibodies had been previously detected had a median time interval to detection of a new antibody of 13 days, with 18 percent of the new antibodies appearing by day 3. Another study corrected immunogenicity calculations based on antibody evanescence and found higher rates of

immunogenicity for antigens, such as Jk^a.²¹ Jk^a also had increased immunogenicity when corrections were made for RBC exposures.²² This finding is an important implication in pre-transfusion testing, especially when antibodies such as anti-Jk^a are found to have a high evanescence rate (61.4% in this study). Additionally, with many new antibodies being identified between days 3 and 13, patients could benefit from set post-transfusion screening protocols during this time, although this testing would increase costs.^{5,23,24}

Considering the previous information, evanescence of antibodies has important implications in pre-transfusion testing. Maintaining the records of alloimmunized patients by hospital transfusion services is crucial, but there is a concern that patients may receive transfusions at more than one hospital. Additionally, a regional or national database of patient antibody findings is recommended. Antigen- or genotype-matching for patients beyond chronic transfusion recipients would prevent alloimmunization and therefore bring benefit to a larger number of transfusion recipients. However, this recommendation is hypothetical, since the discrepancy between the donor population and transfusion recipients does not allow for all patients to receive antigen-matched transfusions.

Because this was a retrospective study, the lack of consistent follow-up could be considered a study limitation having a possible significant impact on antibody detectability. Furthermore, because the patients could be admitted to other hospitals during the follow-up period, there is a probability that the data on blood transfusion and antibodies are not complete. For future studies on antibody evanescence, we recommend a length of follow-up to be at least 5 years. Regarding the number of follow-ups, future studies could focus on two follow-ups after transfusion: one in the first 3 months to detect early-induced antibodies before they disappear and the second in the next 3 months after transfusion to detect late-induced antibodies. For further conclusions, additional studies of patient-specific and peri-transfusion factors on antibody evanescence are needed.

In conclusion, a patient's characteristics (age and sex), results of the initial RBC antibody screen, RBC transfusion, length of follow-up period, and the specificity of the alloantibody(ies) significantly influenced the time of antibody evanescence. In addition to inter-individual patient variability and differences between antigens and their immunogenicity, there are organizational problems with the lack of alloantibody registries for RBCs and no protocols for antibody screening after transfusion or pregnancy. Attempts to prevent alloimmunization

by extended antigen matching are not always feasible because of emergencies and blood shortages. Although mouse models can provide basic answers to immunohematologic questions, there are still biological differences between species, and more reliable models are needed to better understand the behavior of human alloantibodies. In this era of availability of preclinical, clinical, epidemiologic, and translational research, there is still a need for a better understanding of the causes of antibody evanescence, which can be achieved by linking research groups and performing more multidisciplinary studies.

Acknowledgments

We acknowledge all medical doctors and laboratory technicians at the Department of Transfusion Medicine of University Hospital Centre Zagreb who participated in the diagnostics and blood transfusion of alloimmunized patients.

Ethics Statements

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. For this study, written informed consent was not required as decided by the institutional ethical committee (approval number 02/013 AG).

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