BRIEF REPORT

DOI: 10.4274/tjh.galenos.2022.2022.0284
Turk J Hematol 2023;40:50-63

Short-Term Clinical Outcomes of Packed Red Blood Cell Transfusions with Isolated Enzyme-Phase Crossmatch Incompatibility: A Single-Center Cross-Sectional Study

İzole Enzim-Fazı Çapraz Karşılaştırma Uyumsuzluğu Gösteren Kırmızı Kan Hücresi Transfüzyonlarının Kısa Dönem Klinik Sonuçları: Tek Merkezli Kesitsel Bir Çalışma

Umut Yılmaz[1], Alper Yaşar[2], Şeniz Öngören[1], Zafer Başlar[1], Muhlis Cem Ar[1]

[1]*İstanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty, Department of Internal Medicine, Division of Hematology, İstanbul, Türkiye*
[2]*İstanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty, Department of Internal Medicine, İstanbul, Türkiye*

Abstract

Acute hemolytic transfusion reactions (AHTRs) are feared complications of packed red blood cell (PRBC) transfusions. This study aimed to investigate the clinical consequences of isolated enzyme-phase crossmatch-incompatible PRBC transfusions by clinically observing all events during the study period at a single institution with the primary goal of detecting AHTRs. Ninety-four transfusions of interest were administered during the study period. Laboratory investigations were adequate in 73 episodes, where no AHTR developed and a mean hemoglobin concentration rise of 1.1 g/dL was documented. Three transfusions were terminated prematurely; however, further investigations ruled out AHTR. The remaining 21 transfusions were also completed uneventfully without noteworthy clinical deterioration. This study's results provide clinical validation to omit pretransfusion screening with enzyme-phase crossmatch and document the safety and short-term efficacy of isolated enzyme-phase incompatible transfusions. The findings may encourage future clinical research to better understand the long-term efficacy of such transfusions, which may be valuable for transfusion-dependent patients.

Keywords: Transfusion, Crossmatch, Enzyme-phase, Hemolysis, Incompatible crossmatch

Öz

Eritrosit süspansiyonu (ES) transfüzyonlarının korkulan yan etkilerinden birisi akut hemolitik transfüzyon reaksiyonlarıdır (AHTR). Çalışma, izole enzim-fazı çapraz karşılaştırma testi uyumsuz olan ES transfüzyonlarını klinik olarak gözlemleyerek, bu transfüzyonların klinik sonuçlarını araştırmaktadır. Birincil sonlanım noktası, AHTR'lerin saptanmasıdır. Çalışma döneminde araştırmanın kriterlerine uygun 94 transfüzyon yapıldı ve tümü çalışmaya dahil edildi. Bu transfüzyonların 73'ünde yeterli laboratuar incelemesi vardı ve AHTR gelişmediği gösterildi. Bu 73 transfüzyonda ortanca hemoglobin konsantrasyonu artışı 1,1 g/dL oldu. Üç transfüzyon erken sonlandırıldı, fakat ileri incelemelerde AHTR dışlandı. Diğer 21 transfüzyonda yeterli laboratuar incelemesi yapılamasa da bu transfüzyonların sorunsuz tamamlandığı ve klinik takiplerde transfüzyon reaksiyonu düşündürecek klinik bulgu gelişmediği belgelendi. Çalışmanın verileri, enzimli ortamda çapraz karşılaştırmanın transfüzyon öncesi taramada gerekli olmayabileceğinin klinik doğrulaması niteliğindedir ve bu kan ürünlerinin kısa dönemde etkin olduğunu göstermektedir. Özellikle transfüzyona bağımlı hastaların tedavisindeki önemi nedeniyle, izole enzimli ortam uyumsuzluğu olan ES transfüzyonlarının uzun dönem etkinliğinin ileriye dönük kontrollü çalışmalarla araştırılmasını teşvik edici sonuçlar elde edilmiştir.

Anahtar Sözcükler: Transfüzyon, Çapraz karşılaştırma, Enzim fazı, Hemolitik transfüzyon reaksiyonları

Address for Correspondence/Yazışma Adresi: Umut Yılmaz, M.D., İstanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty, Department of Internal Medicine, Division of Hematology, İstanbul, Türkiye
E-mail : umutyza@hotmail.com ORCID: orcid.org/0000-0002-8672-0679

Received/Geliş tarihi: June 27, 2022
Accepted/Kabul tarihi: December 12, 2022

Introduction

Packed red blood cell (PRBC) transfusions expand the blood volume and increase blood oxygen-carrying capacity. Frequently, they are performed as urgent interventions without genuine alternatives and they carry risks of complications. Acute hemolytic transfusion reactions (AHTRs) are life-threatening due to the immediate breakdown of transfused red blood cells. Selecting ABO-compatible donors, antibody screening of recipients, and crossmatch testing considerably reduce the prevalence of AHTRs [1,2].

Antibody detection was improved against some erythrocyte antigens (Rhesus, Lewis, Kidd, I, P) following enzyme manipulation [3,4,5]. However, later studies demonstrated that these tests had low specificity in predicting AHTRs [6,7,8], which raised concerns regarding cost-effectivity and time consumption, reducing their application rates. Physicians face the task of weighing the risks of transfusion reactions against the risks of delaying a possibly life-saving transfusion. In such emergencies, only the risk of the most severe possible transfusion reactions may outweigh the risk of delaying treatment. Even though the specificity of enzyme-phase studies is low, the rate at which they can predict clinically devastating complications may still be significant; thus, real-life data focusing on detecting AHTRs can contribute to clinical decision-making. This study presents our findings regarding the short-term consequences of transfusing PRBCs with isolated enzyme phase crossmatch incompatibility.

Materials and Methods

We designed a study to observe the short-term clinical outcomes of isolated enzyme-phase mismatched PRBC transfusions, which were defined as being blood group-compatible through blood typing and direct anti-human globulin (AHG) crossmatching but incompatible based on enzyme-phase crossmatch studies. Antibody screening and identification were performed only in cases with incompatibility in the AHG-phase cross-matching in accordance with the in-house algorithm for pretransfusion screening. The study's primary aim was to detect short-term adverse events in patients receiving PRBC transfusions with isolated enzyme-phase crossmatch incompatibility, mainly AHTRs. The secondary aim was to assess the efficacy of these transfusions.

The study was conducted with adult PRBC recipients in a single center. Antibody-screening and crossmatch studies were carried out using the gel-card method with bromelain for manipulation as explained in detail in the Supplementary Materials and Methods. The data were collected by two internist physicians within 48 h of the transfusions using a standard transfusion surveillance form. Pre- and posttransfusion laboratory data were extracted from hospital records.

Results

During the study period, 45 adult patients received 94 transfusions with isolated enzyme-phase crossmatch incompatibility, and all cases of such transfusions were included in the study (Table 1). The number of transfusions per patient ranged from 1 to 5. Transfusions were analyzed by categorizing them into four different groups according to whether the transfusions were completed, the setting of the infusion, and the availability of solid follow-up and laboratory data. Ninety-one of 94 transfusions were completed uneventfully (Table 2).

Three transfusions were terminated due to suspected adverse events and these were included in Group I. The laboratory features and clinical progress of these cases are summarized in Table 3 and the Supplementary Materials. After comprehensive evaluations were performed in accordance with blood bank regulations, the premature termination of these transfusions was attributed to fever due to ongoing sepsis, febrile nonhemolytic transfusion reaction, and patient incompliance in the three respective cases.

Table 1. Selected characteristics of the cohort.

Number of patients (n)	45
Gender (female/male)	23/22
Age (years)	Median: 61 (range: 19-83)
Hemoglobin level before transfusion (g/dL)	Median: 7.9 (range: 4.3-10)
Principal causes of anemia	**n**
Perioperative hemorrhage	13
Solid organ malignancy	9
End-stage kidney failure	6
Bleeding from the gastrointestinal tract	6
Hematologic malignancy	4
Trauma	4
Hemoglobinopathy	4
Other	4

Table 2. Group definitions.

Group I	Included transfusion episodes that were prematurely terminated
Group II	Included transfusion episodes among hospitalized patients who stayed in the hospital for more than 48 h after the completion of the infusion
Group III	Included transfusion episodes among hospitalized patients who were discharged within 48 h after the completion of the infusion
Group IV	Included transfusion episodes given in outpatient clinics

Forty-two patients receiving a total of 91 units of PRBCs were analyzed in Groups II-IV. Hemoglobin (Hgb), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) levels as surrogates of efficacy and hemolysis before and within 48 h of the transfusion were available for 73, 64, and 62 of these transfusions, respectively. We documented a median rise of 1.1 g/dL in Hgb levels after the 73 PRBC infusions for which data were available. No significant changes were observed between the median and mean levels of LDH and AST before and after the transfusions (Table 4). Further details on each transfusion episode and each patient are provided in Supplementary Tables 1 and 2, and the outcomes of all groups are summarized in Table 5.

Table 3. Selected laboratory findings of patients in Group I.

Group I	First patient	Second patient	Third patient
Hgb (g/dL)			
Before transfusion	7.2	5.8	7.3
After transfusion	7.1	6.7	7.1
LDH (IU/L)			
Before transfusion	232	159	NA
After transfusion	176	187	494
AST (IU/L)			
Before transfusion	11	7	41
After transfusion	12	9	35
Haptoglobin (mg/dL)			
After transfusion	319	277	36

Hgb: Hemoglobin; LDH: lactate dehydrogenase; AST: aspartate aminotransferase, NA: not available.

Table 4. Summary of laboratory parameters for Group II.

Parameter	Pretransfusion	Posttransfusion
Hemoglobin (g/dL) n=73 transfusions	Median: 8/range: 4.3-10.7 Mean: 8.1/SD: 1.24	Median: 9.2/range: 5.7-12.3 Mean: 9.4/SD: 1.31
LDH (IU/L) n= 64 transfusions	Median: 255/range: 143-785 Mean: 303.5/SD: 140.1	Median: 235/range: 125-705 Mean : 293.2/SD: 138.3
AST (IU/L) n=50 transfusions	Median: 26.5/range: 12-173 Mean: 40.0/SD: 34.0	Median: 24/range: 9-191 Mean: 39.3/SD: 39.6

LDH: Lactate dehydrogenase; AST: aspartate aminotransferase; SD: standard deviation.

Table 5. Summary of outcomes for each analyzed group.

Group	Number of patients	Number of transfusions	Adverse events
I	3	3	1 FNHTR AHTR did not develop
II	30	68	AHTR did not develop
III	8	9	AHTR not detected
IV	9	14	AHTR not detected

FNHTR: Febrile nonhemolytic transfusion reaction; AHTR: acute hemolytic transfusion reaction.

Group II consisted of 30 patients who received a total of 68 units of PRBCs. These patients were adequately observed to decide whether significant acute transfusion reactions occurred or not as they remained hospitalized for over 48 h after the transfusions and each case was thoroughly followed and analyzed by the researchers. All 68 transfusions were completed without interruption. No transfusion-related complications were identified in clinical assessments and follow-up. Significant LDH elevation was found in three cases and attributed to sternotomy in two cases and tumor lysis syndrome in the other (transfusions #22, #54, and #71 - Supplementary Tables).

Nine PRBC transfusions were analyzed in Group III. The patients in this group were discharged from the hospital within 48 h of the transfusions. Thus, follow-up data and posttransfusion laboratory testing results were unavailable in most cases. All transfusions in this group were completed without any alarming events. We found no signs of transfusion-related complications within the range of the available data.

Group IV included 14 transfusions performed at outpatient clinics as transfusion support for chronic diseases. These patients were seen only once by the researchers. No reactions were detected or reported in these patients during or after the transfusions. Through hospital records, we confirmed that these patients resumed their regular outpatient care in the following months.

Discussion

Improved detection of antibodies against Rh, Kidd, and Lewis systems following enzyme manipulation has been shown in previous studies [3,4], suggesting that enzyme-phase screening can prevent rare hemolytic reactions. However, later studies concluded that enzyme-phase antibody screening had low specificity [6]. Castellá et al. [11] studied 1070 plasma samples with antibodies detectable only through enzyme-phase screening and reported that only 0.6% of these samples had antibodies directed to a known erythrocyte antigen. Similar results were reported by Enko et al. [8], who studied 2420 plasma samples and recommended the exclusion of enzyme-phase screening from routine use.

Although the studies mentioned above provided valuable information at the laboratory level, there are limited data concerning the clinical consequences of isolated enzyme-phase incompatible PRBC transfusions. From the physician's point of view, severe transfusion reactions that result in rapid clinical deterioration of the recipient are the most important, especially when a transfusion is urgently needed. Low specificity does not reassure the physician if the rare cases of true positivity entail devastating complications. In this context, our study is informative and unique in that it directly considers the clinical outcomes of isolated enzyme-phase incompatible PRBC

transfusions with a focus on individual patients and episodes. We provide the real-life clinical data of all 45 patients transfused with isolated enzyme-phase incompatible PRBCs in a 10-month period at a single institution where no severe acute transfusion reactions developed. Our clinical observations, as reported here, suggest that pretransfusion enzyme-phase compatibility testing may be overly time-consuming and better avoided in emergency settings.

Delayed hemolytic transfusion reactions (DHTRs) increase the frequency of transfusion need in patients with transfusion dependence, which further complicates iron overload and can trigger sickle cell crises [13]. Our findings of short-term efficacy and safety imply that it would be helpful to perform erythrocyte survival studies to understand the value of enzyme-phase testing to detect DHTRs [14], which may be crucial for transfusion-dependent patients.

The major limitation of this study is its observational nature, which precluded the preparation of controlled conditions. As a result, the content and timing of laboratory tests were not homogeneous. Bromelain was used in our center for erythrocyte manipulation and our data may be inapplicable for other enzymes. Due to the noninterventional design of the study, antibody screening and antibody identification results were unavailable as these were not routinely performed according to the pretransfusion screening algorithm of the center if the AHG-phase cross-matching was compatible. Thus, even though we carefully analyzed the data of each case individually to detect adverse reactions, our conclusions in selected cases were no better than expert opinions. Given all these limitations, our findings must be interpreted with caution.

Conclusion

Our data on 94 episodes of PRBC transfusions with isolated enzyme-phase crossmatch incompatibility suggest that such transfusions are not associated with overt acute hemolytic reactions. Isolated enzyme-phase crossmatch-incompatible transfusions resulted in effective increases in mean Hgb. This study's results do not provide evidence to support the routine use of enzyme-phase crossmatching as a pretransfusion screening method for urgent transfusions. The results are encouraging and further studies on this subject are warranted, especially to detect the long-term efficacy of these transfusions in transfusion-dependent patients.

Ethics

Ethics Committee Approval: The İstanbul University-Cerrahpaşa Cerrahpaşa Medical Faculty's Clinical Research Ethics Committee granted approval for the research titled "Short-Term Clinical Outcomes of Packed Red Blood Cell Transfusions with Isolated Enzyme-Phase Crossmatch Incompatibility: A Single-Center Study" on October 3, 2017, with approval number 16804004-604.01.02-339411.

Authorship Contributions

Concept: U.Y.; Design: M.C.A.; Data Collection or Processing: A.Y.; Analysis or Interpretation: Z.B., M.C.A.; Writing: Ş.Ö., Z.B.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Landsteiner K. Agglutinationserscheinungen normalen menschlichen Blutes. 1901 [Agglutination phenomena of normal human blood]. Wien Klin Wochenschr 2001;113:768-769.
2. Beck ML, Tilzer LL. Red cell compatibility testing: a perspective for the future. Transfus Med Rev 1996;10:118-130.
3. Dodd BE, Eeles DA. Rh antibodies detectable only by enzyme technique. Immunology 1961;4:337-345.
4. Hill BC, Hanna CA, Adamski J, Pham HP, Marques MB, Williams LA 3rd. Ficin-treated red cells help identify clinically significant alloantibodies masked as reactions of undetermined specificity in gel microtubes. Lab Med 2017;48:24-28.
5. Berthier MG, Woo LJ. Detection of atypical antibodies in erythrocytes; practical experience with the papain-treated erythrocyte agglutination and indirect antiglobulin technics. Am J Clin Pathol 1954;24:1419-1423.
6. Issitt PD, Combs MR, Bredehoeft SJ, Campbell ML, Heimer M, Joyner L, Lorentsen L, Remley C, Bullock S, Bumgarner J, Zakeriniasar M, Kirkland AW, Melroy HM, Millikin DM. Lack of clinical significance of "enzyme-only" red cell alloantibodies. Transfusion 1993;33:284-293.
7. Pereira A, Mazzara R, Gelabert A, Castillo R. Two-stage papain-indirect antiglobulin test and LISS direct agglutination are not appropriate for pretransfusion screening for unexpected antibodies. Haematologica 1991;76:475-478.
8. Enko D, Habres C, Wallner F, Mayr B, Halwachs-Baumann G. Frequencies and specificities of "enzyme-only" detected erythrocyte alloantibodies in patients hospitalized in Austria: is an enzyme test required for routine red blood cell antibody screening? J Blood Transfus 2014;2014:532919.
9. Wiesen AR, Hospenthal DR, Byrd JC, Glass KL, Howard RS, Diehl LF. Equilibration of hemoglobin concentration after transfusion in medical inpatients not actively bleeding. Ann Intern Med 1994;121:278-230.
10. Elizalde JI, Clemente J, Marín JL, Panés J, Aragón B, Mas A, Piqué JM, Terés J. Changes in hemoglobin and hematocrit levels after packed red cell transfusion in patients with acute anemia. Transfusion 1997;37:573-576.
11. Castellá D, Cid J, Panadés M, Martín-Vega C. One thousand seventy antibodies detected only by a 2-stage papain test: wanted and unwanted positive reactions. Immunohematology 2001;17:122-124.
12. Koseoglu M, Hur A, Atay A, Cuhadar S. Effects of hemolysis interferences on routine biochemistry parameters. Biochem Med (Zagreb) 2011;21:79-85.
13. Gardner K, Hoppe C, Mijovic A, Thein SL. How we treat delayed haemolytic transfusion reactions in patients with sickle cell disease. Br J Haematol 2015;170:745-756.
14. Mock DM, Widness JA, Veng-Pedersen P, Strauss RG, Cancelas JA, Cohen RM, Lindsell CJ, Franco RS. Measurement of posttransfusion red cell survival with the biotin label. Transfus Med Rev 2014;28:114-125.

Supplementary Table 1. Selected features of transfusion episodes.

Transfusion #-patient #[1]	Timing[2]	Body temperature (°C)	BP-HR	RR	Hgb (g/dL)	LDH (IU/L)	AST (IU/L)	Indirect bilirubin (mg/dL)	Premedication	Group/ comments
1-1	Pre-t Post-t	36.8 36.3	90/60-73 100/60-78	16 16	7.8 8.5	333 260	29 28	0.5 0.6	None	II/no complications
2-1	Pre-t Post-t	35.8 36.3	90/60-73 90/60-78	16 16	8.5 9	260 265	28 27	0.6 0.6	None	II/no complications
3-1	Pre-t Post-t	36.4 37.0	90/60-80 90/60-78	16 16	9 10.1	265 235	27 31	0.6 0.7	None	II/no complications
4-2	Pre-t Post-t	36.5 37.2	100/60-92 110/70-91	18 16	8.2 8.8	210 161	10 NA	NA 0.1	MP, 40 mg	II/no complications
5-2	Pre-t Post-t	37.5 37.3	90/60-105 100/70-101	16 16	8.8 9.4	NA NA	NA NA	NA NA	None	III/no complications
6-3	Pre-t Post-t	36.9 36.6	125/73-98 132/69-92	20 20	7.7 8.9	164 206	36 27	0.1 0.1	AH	II/no complications
7-3	Pre-t Post-t	36.1 37	130/60-106 130/60-104	17 19	7.4 9	207 187	31 30	0.2 0.1	None	II/no complications
8-3	Pre-t Post-t	36.8 36.9	127/78-100 125/75-98	20 20	7.8 8.7	184 190	23 15	0.1 0.05	None	II/no complications
9-4	Pre-t Post-t	35.6 36	150/70-72 150/80-86	22 22	6.5 7.6	785 688	116 98	0.1 0.1	None	II/no complications, LDH and AST elevated due to severe myositis (pre-t CK: 3833 IU/L)
10-4	Pre-t Post-t	36.4 36.2	145/70-81 140/80-74	18 20	6.5 7.8	684 705	72 61	0.2 0.2	None	II/no complications
11, 12-5	Pre-t Post-t	36.8 37	93/60-73 110/71-66	20 20	4.3 5.7	203 161	26 24	0.5 0.4	None	II/no complications, 2 units of PRBCs administered consecutively without interruption
13-5	Pre-t Post-t	36.2 35.7	105/72-65 100/64-66	20 20	5.7 7	143 145	25 20	0.2 0.2	None	II/no complications
14, 15-6	Pre-t Post-t	36.3 36.4	130/80-73 130/80-75	20 20	8.8 10.9	NA NA	18 9	0.2 NA	None	II/no complications, post-t centrifuged plasma had no red discoloration excluding extravascular hemolysis, 2 units of PRBCs administered consecutively without interruption

16-7	Pre-t Post-t	36 36.4	130/80-66 130/80-58	20 22	9.3 10.9	NA NA	34 18	NA NA	None	II/no complications, post-t centrifuged plasma had no red discoloration excluding extravascular hemolysis
17-7	Pre-t Post-t	35.9 35.8	175/84-62 144/72-55	16 14	8.6 10.4	NA NA	NA NA	NA NA		III/no complications, discharged 8 h after this transfusion
18-8	Pre-t Post-t	36.4 36.5	160/60-84 150/60-64	16 16	9 10.1	214 230	15 18	0.1 0.1	None	II/no complications
19-8	Pre-t Post-t	36.3 36.9	167/73-61 167/65-61	16 16	9.2 10.6	255 231	20 NA	0.1 NA	None	II/no complications
20-9	Pre-t Post-t	35.7 36.7	80/43-95 120/70-84	24 20	7.6 9.2	482 NA	24 NA	0.2 NA	None	IV/no complications
21-10	Pre-t Post-t	35.5 36.0	144/92-96 152/99-90	22 22	10.5 11.3	183 NA	33 NA	0.2 NA	None	III/no complications
22, 23-11	Pre-t Post-t	24 24	80/50-70 80/50-70	MV MV	9.9 8.9	232 445	21 54	0.2 0.2	NA	II/transfusions were performed during surgery (CABG) where the estimated blood loss was 1.5 L, plasma of centrifuged post-t samples was bright yellow
24-11	Pre-t Post-t	35.7 36.7	110/60-120 120/80-105	MV MV	8.9 9.6	445 308	54 28	0.2 0.2	None	II/no complications
25, 26-11	Pre-t Post-t	36.8 37.2	120/70-110 100/50-105	MV MV	9.6 12.1	308 300	28 31	0.2 0.2	None	II/no complications, 2 units of PRBCs administered consecutively
27-12	Pre-t Post-t	36 36	140/70-89 120/70-83	16 16	7.3 8.4	161 125	15 10	0.1 0.2	MP, 20 mg	II/no complications
28-13	Pre-t Post-t	36.4 36.7	110/70-96 120/60-102	22 24	8 9.1	297 324	19 24	0.5 0.5	None	II/no complications
29, 30-14	Pre-t Post-t	36.7 36.6	123/67-86 138/70-81	20 20	6.7 10.4	375 320	48 39	0.2 0.3	MP + AH	II/no complications, 2 units of PRBCs administered consecutively
31-15	Pre-t Post-t	37.2 37	130/80-88 120/70-104	18 18	6.7 NA	280 NA	11 NA	0.6 NA	None	IV/no complications, 1 week later Hgb was 7.2 g/dL
32-15	Pre-t Post-t	36.6 36.4	120/60-92 110/60-94	18 18	7.2 NA	NA NA	NA NA	NA NA	None	IV/no complications, 1 week later Hgb was 7.7 g/dL
33-16	Pre-t Post-t	37 37.3	100/60-72 100/70-64	24 24	7.7 8.7	175 NA	25 11	0.8 0.3	None	II/no complications

34-17	Pre-t Post-t	36.6 37.2	110/60-72 110/70-77	22 22	5.9 6.5	569 539	18 13	2.5 1.2	None	II/no complications
35-17	Pre-t Post-t	36.4 36.6	105/50-65 110/60-65	20 20	6.5 7.8	539 580	13 15	1.2 1.5	None	II/no complications
36-17	Pre-t Post-t	36.9 36.8	90/60-72 110/50-65	20 20	7.8 NA	NA NA	NA NA	NA NA	None	IV/no complications
37-17	Pre-t Post-t	36.3 36.4	100/60-77 120/60-75	16 16	8.2 NA	NA NA	NA NA	NA NA	None	IV/no complications
38-18	Pre-t Post-t	37.3 37.3	100/70-112 140/90-113	20 20	8.4 9.2	489 314	60 55	0.3 0.2	None	II/no complications
39-18	Pre-t Post-t	36.8 36.5	130/60-96 130/50-99	16 16	8.7 9.4	392 336	12 14	0.2 0.1	None	II/no complications
40-19	Pre-t Post-t	36.2 36.2	100/60-105 100/60-96	20 20	7.8 NA	435 NA	24 NA	1 NA	None	IV/no complications
41, 42-20	Pre-t Post-t	36.7 37.0	114/72-85 112/66-88	14 14	10.7 12.3	351 NA	25 18	0.8 0.8	None	II7/no complications, 2 units of PRBCs administered during surgery
43-21	Pre-t Post-t	36.5 36.0	134/91-112 127/90-101	22 22	5.9 NA	350 NA	40 NA	1.1 NA	None	IV/no complications
44, 45, 46, 47-22	Pre-t Post-t	36.7 37.3	105/71-90 106/78-101	20 24	7.7 12.1	296 170	23 20	0.5 0.6	None	II/no complications, 4 units of PRBCs administered consecutively
48-23	Pre-t Post-t	36.5 35.9	144/87-62 151/99-61	20 20	9.2 NA	252 NA	15 NA	0.3 NA	None	III/no complications
49, 50-24	Pre-t Post-t	37.0 36.6	92/53-94 99/51-93	16 16	5.9 NA	NA NA	NA NA	NA NA	None	III/no complications
51-25	Pre-t Post-t	36.2 36.6	110/70-115 110/70-108	24 24	6.2 8.1	323 209	14 13	0.2 0.2	MP	II/no complications
52, 53-25	Pre-t Post-t	36.1 36.0	120/80-98 130/80-96	22 22	8.1 10.1	209 214	13 18	0.2 0.2	MP	II/no complications
54, 55-26	Pre-t Post-t	37.4 37.5	85/60-118 90/60-107	24 24	7 10	331 529	40 140	0.1 0.1		II/started on cytotoxic chemotherapy the same day with 2 units of PRBC transfusions; centrifuged post-t sample was bright yellow, Hgb rose effectively and remained above 9 g/dL for 10 days; elevated LDH/AST ascribed to TLS
56-27	Pre-t Post-t	36 37	150/90-103 170/90-109	28 28	8.5 9.3	200 216	14 16	0.1 0.1	MP, 20 mg	II/no complication

57-27	Pre-t Post-t	36.6 36.9	150/80-84 180/90-92	28 28	7.9 9.2	NA NA	25 16	0.1 0.1	AH	II/no complications, centrifuged post-t sample was bright yellow
58-27	Pre-t Post-t	36.6 36.1	180/100-92 180/60-95	28 28	9.2 10	NA NA	NA NA	NA NA	None	III/no complications
59-27	Pre-t Post-t	36.1 36.6	150/90-78 150/80-83	24 24	8.6 10.1	216 231	NA NA	0.1 0.1	None	II/no complications
60-27	Pre-t Post-t	36.0 35.8	160/80-99 170/90-81	28 28	7.6 9.2	236 214	16 13	0.1 0.1	None	II/no complications
61, 62-28	Pre-t Post-t	36 36.7	90/50-72 120/70-64	12 12	7.2 NA	385 NA	25 NA	3.6 NA	None	IV/no complications
63, 64-28	Pre-t Post-t	36.5 36.7	110/50-66 100/60-70	12 12	7.7 NA	NA NA	NA NA	NA NA	None	IV/no complications
65-29	Pre-t Post-t	36.6 36.5	157/80-79 159/82-84	18 18	8.1 NA	265 NA	26 NA	0.2 NA	None	III/no complications
66-29	Pre-t Post-t	36.5 36.8	160/6072 170/80-80	18 18	7.7 9	204 171	20 17	0.2 0.2	None	II/no complications
67, 68, 69-30	Pre-t Post-t	36.5 36.9	170/90-95 160/80-96	24 21	5.8 10.3	206 216	13 13	0.4 0.3	Dexamethasone + AH	II/no complications, 3 units of PRBCs administered consecutively
70-31	Pre-t Post-t	37 36.9	161/95-76 140/90-78	14 14	7.4 8.7	192 208	50 23	0.2 0.5	None	II/no complications
71-32	Pre-t Post-t	38 37.5	140/80-80 130/70-80	MV MV	8 9	173 390	48 87	0.2 0.4	Surgery	II/transfused during CABG surgery, plasma of centrifuged post-t samples was bright yellow, CK was highly elevated
72-32	Pre-t Post-t	37.2 37.2	125/75-70 130/70-65	MV MV	9 9.6	390 389	87 53	0.4 0.1	None	II/no complications
73-32	Pre-t Post-t	36.6 36.1	110/65-90 130/65-80	24 25	9.6 10.8	389 393	53 34	0.1 0.2	None	II/no complications, patient recovered following surgery and was discharged 1 week after the last transfusion with Hgb of 10.2 g/dL and LDH 282 IU/L
74-33	Pre-t Post-t	37.1 37	140/50-70 130/60-74	24 24	7.4 8.3	237 213	14 15	0.2 0.1	None	II/no complications
75-33	Pre-t Post-t	36.4 37	150/60-66 140/60-64	20 20	7.3 8.2	212 241	12 13	0.1 0.1	None	II/no complications
76-34	Pre-t Post-t	37.1 36.9	117/68-96 109/60-93	20 20	8 8.2	301 169	110 79	3.1 2.8	None	II/no complications
77-34	Pre-t Post-t	35.9 3	110/64-104 117/77-99	24 24	7.7 8.6	178 192	127 173	1.7 2.6	None	II/no complications

78-34	Pre-t Post-t	35.4 35.7	100/65-80 110/80-85	24 24	8.6 9.7	192 261	173 191	2.6 2.2	None	II/no complications
79-35	Pre-t Post-t	36.1 35.8	93/62-105 87/61-101	12 12	7.3 NA	NA NA	NA NA	NA NA	None	IV/no complications
80-36	Pre-t Post-t	33 33	80/50-112 100/60-120	MV MV	9.8 8.8	424 425	87 60	0.5 0.6	None	II/no complications, transfused during CABG surgery for acute coronary syndrome, plasma of centrifuged post-t samples was bright yellow, CK and troponin-T were highly elevated
81-36	Pre-t Post-t	33 34	109/46-129 120/55-130	MV MV	8.8 9.8	425 438	60 55	0.6 0.4	None	II/no complications, transfused during CABG
82-36	Pre-t Post-t	36.1 36	120/50-90 130/60-85	22 20	8.8 10.8	438 335	55 38	0.4 0.5	None	II/no complications
83-36	Pre-t Post-t	36 35.7	110/40-100 100/50-91	16 16	10.6 12.3	357 357	62 82	0.9 0.7	None	II/no complications
84-37	Pre-t Post-t	37.2 37.0	180/90-65 180/60-62	14 14	8.6 9.9	335 NA	NA NA	0.8 NA	MP, 20 mg	III/no complications
85-38	Pre-t Post-t	36.2 35.9	102/61-86 99/57-81	12 12	8.6 NA	261 NA	66 NA	1.8 NA	None	IV/no complications (exchange transfusion)
86, 87-39	Pre-t Post-t	36 36.3	110/80-80 110/70-84	20 20	7.6 9.1	201 164	20 21	0.3 0.4	AH	II/no complications, 2 units of PRBCs administered consecutively
88-40	Pre-t Post-t	36 36.7	135/81-90 135/85-95	20 20	8.9 10.1	224 181	33 34	0.4 0.3	None	II/no complications
89, 90-41	Pre-t Post-t	36 36.7	135/81-90 135/85-95	20 20	7.5 9.7	588 NA	14 21	0.1 0.05	None	II/no complications, 2 units of PRBCs administered consecutively
91-42	Pre-t Post-t	35.8 35.7	125/70-79 115/65-77	16 16	7.5 NA	312 NA	12 NA	0.6 NA	None	IV/no complications

[1]Each unit of packed red blood cells (PRBCs) is assigned to a number label. Each row in Supplementary Table 1 represents the data concerning the infusion of the indicated PRBCs. Patient #indicates the patient (as detailed in Supplementary Table 2) for whom the PRBCs in question were applied. Numbered labels are consistent between Supplementary Tables 1 and 2.

[2]Pre-t (pretransfusion) data reflect vital signs just before the onset of the given transfusion and laboratory data up to 24 h before the onset of transfusion. Post-t (posttransfusion) data reflect vital signs just after the transfusion was completed and laboratory data within 24 h after the completion of transfusion. Vital signs were documented every 15 min during transfusions. Among the entire cohort, only the three patients in Group I (see main text) had significant alterations in vital signs during the course of transfusion.

BP: Blood pressure (mmHg); HR: heart rate (beats per minute); RR: respiratory rate (per minute); MV: mechanical ventilation; CABG: coronary artery bypass grafting; Hgb: hemoglobin; LDH: lactate dehydrogenase; AST: aspartate aminotransferase; NA: not available; MP: methylprednisolone; PRBC: packed red blood cell; AH: antihistaminic; CK: creatine kinase; TLS: tumor lysis syndrome.

Supplementary Table 2. Selected characteristics of each patient in the cohort.

Patient #[1]	Transfusion (s) #[1]	Age/gender	Medications	Comorbidities	Prior transfusion/ pregnancy	Cause of anemia	Comments
1	1, 2, 3	62/M	Warfarin, metoprolol, colchicine, PPI, allopurinol, spironolactone, furosemide, cefoxitin	CHF, gout, AF, MVR	Yes (2014)/ no	Hemorrhagic pleural effusion	No complications
2	4, 5	77/F	Tramadol, enoxaparin, PPI, citalopram, atorvastatin, metoprolol, betahistine, piracetam	CAD, HT, nephrectomy, thyroidectomy	No/yes	Bleeding during orthopedic surgery	Discharged 1 day after transfusion
3	6, 7, 8	62/M	Enoxaparin, fentanyl, tramadol, ceftazidime, pemetrexed, zoledronate	Stage 4 lung carcinoma	Yes (2017)/ no	Chronic inflammation, hemoptysis	No complications
4	9, 10	70/F	Metoprolol, amlodipine, sertraline, tramadol, prednisolone, enoxaparin, allopurinol, PPI	Dermatomyositis, CKD, HT, CHF	No/yes	Chronic inflammation, CKD	No complications, LDH and AST elevated due to myositis
5	11, 12, 13	61/M	Azathioprine, prednisone, metoprolol, bicarbonate, lercanidipine, PPI, allopurinol, meropenem, glargine	DM, CKD, renal transplantation, HT	No/no	CKD	No complications
6	14, 15	66/M	Leflunomide, infliximab, methylprednisolone, lercanidipine, PPI, tramadol, metoclopramide	Rheumatoid arthritis, HT	No/yes	Chronic inflammation, surgical bleeding	No complications
7	16, 17	66/F	Cefazolin, tramadol, PPI, tranexamic acid	Chronic HBV, cirrhosis	No/yes	Cirrhosis, surgical bleeding	No complications
8	18, 19	77/F	Metoprolol, allopurinol, olmesartan, warfarin	HT, CHF, aortic valve repair	Yes/yes	GI bleeding	No complications
9	20	79/M	Losartan, hydrochlorothiazide, amlodipine, rivastigmine, valacyclovir, Bactrim, terazosin	AML, HT, dementia	Yes/no	AML	No complications
10	21	65/M	Cefazoline	Stage 4 urothelial carcinoma	No/no	Neurosurgical bleeding	No complications
11	22, 23, 24, 25, 26	73/F	Dopamine, heparin, isoniazid, rifampicin, bisoprolol, candesartan, isosorbide, clopidogrel, aspirin, atorvastatin, PPI, trimetazidine	CAD, HT, CHF	No/yes	Bleeding during cardiac surgery	No complications
12	27	82/M	Ramipril, gliclazide, quetiapine, PPI, vitamin B12	DM, HT, gastric adenocarcinoma	No/no	Bleeding during gastrectomy	No complications
13	28	66/M	TPN, imipenem, teicoplanin, metoclopramide, enoxaparin, amikacin, tigecycline, human albumin, ondansetron, furosemide	Stage 4 colon cancer	Yes/no	Chronic inflammation, GI bleeding	No complications
14	29, 30	66/F	Ceftriaxone, ramipril, amlodipine, PPI, gemcitabine, carboplatin, granisetron	Stage 4 ovarian cancer, CKD	Yes/yes	CKD, surgical bleeding	No complications

15	31, 32	64/M	Valacyclovir, moxifloxacin, Bactrim, candesartan, hydrochlorothiazide, MP, allopurinol, rituximab	CLL, PRCA, HT	Yes/no	CLL-PRCA	Received regular transfusions, no complications
16	33	28/F	Clarithromycin, PPI, ondansetron, salbutamol, paracetamol	-	No/no	Hemothorax	No complications
17	34, 35, 36, 37	66/M	Hydroxyurea, ruxolitinib, spironolactone, furosemide, tranexamic acid, tamsulosin	BPH, primary myelofibrosis	Yes/no	GI bleeding, PMF	No complications, receives regular transfusion support, hemolytic markers routinely elevated due to ineffective erythropoiesis, reticulocytes of <1%
18	38, 39	59/F	Captopril, cyclosporine, prednisone, aspirin, enoxaparin, furosemide, piperacillin/tazobactam, metoclopramide, ranitidine	Membranous nephropathy, stage 4 ovarian cancer	No/yes	CKD, surgical bleeding	No complications
19	40	67/F	Azathioprine, ibandronate, prednisone, aspirin	SLE, hemolytic anemia	No/yes	Hemolytic anemia	No complications
20	41, 42	32/M	PPI, ceftriaxone	Trauma	No/no	Trauma	No complications
21	43	56/M	PPI, metformin, rosuvastatin, nateglinide, rituximab	DM, HCL	No/no	HCL	No complications
22	44, 45, 46, 47	57/F	PPI, candesartan, palonosetron, carboplatin, paclitaxel	Stage 4 ovarian cancer	No/yes	Severe surgical bleeding	No complications
23	48	80/F	Metoprolol, rivaroxaban, diltiazem, isosorbide, bevacizumab, oxycodone	AF, HT, DM, stage 4 ovarian cancer	Yes/yes	Chronic inflammation	No complications
24	49, 50	30/F	Folic acid, penicillin	Thalassemia, splenectomy	Yes/no	Thalassemia	No complications
25	51, 52, 53	61/F	Piperacillin tazobactam, PPI, metoclopramide, capecitabine, oxaliplatin, metoprolol, ramipril, hydrochlorothiazide	HT, gastric adenocarcinoma	No/yes	GI bleeding	No complications
26	54, 55	28/M	Ipratropium, allopurinol, PPI, valacyclovir, dexamethasone, fentanyl, granisetron, tenofovir, fluconazole, ifosfamide, etoposide	Metastatic sarcoma	No/no	Chronic inflammation, myelosuppression	Mild TLS
27	56, 57, 58, 59, 60	58/F	Insulin aspart, glargine, metoprolol, levetiracetam, allopurinol, furosemide, enoxaparin, amiodarone, L-thyroxine, atorvastatin, phenytoin, nifedipine, rilmenidine, mannitol	DM, ESRD, CHF, hypothyroidism, epilepsy	Yes/yes	CKD, iron deficiency	No complications
28	61, 62, 63, 64	39/M	Folic acid, deferasirox, aspirin	Thalassemia, splenectomy, haemochromatosis	Yes/no	Thalassemia	No complications, receives regular transfusions

29	65, 66	76/F	Metoprolol, clopidogrel, insulin NPH, amlodipine, rivaroxaban	DM, HT, CKD, AF	Yes/yes	ESRD	No complications
30	67, 68, 69	78/F	Furosemide, amlodipine, metformin, captopril, enoxaparin	CHF, DM, HT, stroke	No/yes	Trauma	No complications
31	70	54/M	Albendazole, NSAID, ceftriaxone, metoclopramide	Liver hydatic cyst	No/yes	Surgical bleeding, GI bleeding	No complications
32	71, 72, 73	74/F	Citalopram, quetiapine, furosemide, propofol, dopamine, amiodarone, tinzaparin, isosorbide, cefazoline, aspirin	CHF, CAD, acute coronary syndrome, anxiety disorder	No/yes	Surgical bleeding	No complications
33	74, 75	79/M	PPI, citalopram, nifedipine, glargine, aspirin, L-thyroxine, lispro, allopurinol, tolvaptan, piperacillin/tazobactam	ESRD, CHF, DM, hypothyroidism	Yes/no	ESRD	No complications
34	76, 77, 78	57/M	Ceftriaxone, metronidazole, NSAID, PPI	Cholangiocellular carcinoma	Yes/no	Chronic inflammation, malnutrition	No complications
35	79	34/M	Folic acid, PPI, deferasirox, growth hormone, testosterone, L-thyroxine	Thalassemia, hemochromatosis, hypopituitarism	Yes/no	Thalassemia	No complications
36	80, 81, 82, 83	73/M	PPI, tranexamic acid, protamine, amiodarone, metoprolol, pancreatic enzyme concentrates	AF, CHF, renal cell carcinoma nephrectomy, CAD	Yes/no	Surgical bleeding, chronic inflammation	No complications
37	84	64/F	Warfarin, oral iron, irbesartan, penicillin, PPI, metformin	Aortic valve replacement, DM, rheumatic fever	Yes/yes	Iron deficiency, valve hemolysis	No complications
38	85	34/F	Folic acid, aspirin, deferasirox, hydroxyurea	Sickle cell anemia, hemochromatosis	Yes/yes	Sickle cell disease	No complications
39	86, 87	41/M	Ceftriaxone, metronidazole, PPI, metoclopramide, tranexamic acid, alginate, vitamin K	Duodenal ulcer	No/no	GI bleeding	No complications
40	88	63/F	Zofenopril, vildagliptin, metformin, rosuvastatin, aspirin, ursodeoxycholic acid	DM, HT, cholelithiasis	No/yes	Undiagnosed (transfused before cholecystectomy, discharged without evaluation of anemia)	No complications
41	89, 90	50/F	Cefazoline, metronidazole, metformin, enoxaparin, vitamin concentrates	DM, stage 4 endometrial carcinoma	No/yes	Surgical bleeding	No complications
42	91	66/M	Mycophenolate, deferasirox, PPI, tamsulosin, aspirin	PRCA, BPH	Yes/no	PRCA	No complications

[1]Each patient is assigned a number label. Each row in Supplementary Table 2 presents the data regarding the given patient. Transfusion # indicates the number labels of the PRBCs (detailed in Supplementary Table 1) that the patient in the given row received. Numbered labels are consistent between Supplementary Tables 1 and 2.

M: Male, F: female; PPI: proton pump inhibitor; CHF: congestive heart failure; AF: atrial fibrillation; MVR: mitral valve replacement; CAD: coronary artery disease; HT: hypertension; CKD: chronic kidney disease; DM: diabetes mellitus; LDH: lactate dehydrogenase; AST: aspartate aminotransferase; HBV: hepatitis B virus; AML: acute myeloid leukemia; GI: gastrointestinal; MP: methylprednisolone; CLL: chronic lymphocytic leukemia; PRCA: purer red cell aplasia; BPH: benign prostatic hyperplasia; PMF: primary myelofibrosis; SLE: systemic lupus erythematosus; HCL: hairy cell leukemia; TLS: tumor lysis syndrome; ESRD: end-stage renal disease; NPH: neutral protamine Hagedorn; NSAID: nonsteroidal anti-inflammatory drug.

Supplementary Materials and Methods

We designed a study to observe the short-term clinical outcomes of packed red blood cell (PRBC) transfusions that were blood group-compatible through direct crossmatching but incompatible in enzyme-phase crossmatch studies. The study's primary aim was to detect short-term adverse events in patients receiving PRBC transfusions with isolated enzyme-phase crossmatch incompatibility, mainly acute hemolytic transfusion reactions (AHTRs). The secondary aim was to assess the efficacy of these transfusions.

The study was conducted with adult PRBC recipients in the İstanbul University-Cerrahpaşa Cerrahpaşa Medical Faculty between November 2017 and August 2018. The Cerrahpaşa Medical Faculty's Clinical Research Ethics Committee granted approval for the research on October 3, 2017, with approval number 16804004-604.01.02-339411. The pretransfusion screening policy for PRBC transfusions in this institution involves crossmatch studies before every transfusion with both direct incubation and an additional incubation following enzyme manipulation of the donor erythrocytes. Antibody screening and crossmatch studies were carried out using the gel-card method of the ORTHO AutoVue Innova System manufactured by Johnson & Johnson (USA; serial no: 4829). Bromelain enzyme was used for erythrocyte manipulation. According to institutional policies, patients receiving an isolated enzyme-phase crossmatch-incompatible PRBC transfusion are managed through hematology consultation. In the event of isolated enzyme-phase crossmatch incompatibility, repeated crossmatches are requested with other PRBCs to find a compatible product. However, in life-threatening conditions and time-sensitive emergencies, or if fully compatible PRBCs are not available, the in-house protocols recommend transfusion of PRBC products with isolated enzyme-phase crossmatch incompatibility under the guidance of a hematology consultant and careful observation of the patient for signs of hemolysis through biochemical markers obtained both before and after the transfusion.

The data were collected by two physicians with over 3 years of experience in internal medicine, and all patients underwent consultation with hematologists. The blood bank staff notified the investigators each time a PRBC with pretransfusion screening results that met the criteria for the study would be released for transfusion. Data on the recipient's age, sex, blood group, underlying diseases, transfusion indication, physical examination results, prior transfusion history, prior surgeries, prior pregnancies, current medications, pretransfusion screening results, and laboratory tests, as well as information on the course of transfusion, were collected from the recipients in person and from electronic patient files within 48 h of the transfusion using a standard transfusion study form. Inpatients were followed with daily visits. Pre- and posttransfusion complete blood counts, hemolysis parameters, electrolytes, and liver and kidney function test results were obtained from the patients' medical files. Additional diagnostic work-ups, including antibody screening, antibody identification, blood cultures, and further testing for monitoring hemolysis, were done in cases of suspected transfusion reactions.

Detailed Clinical Progress of the Patients in Group I

The first patient in Group I was a 30-year-old female with a diagnosis of familial polyposis coli being treated for a pelvic abscess following colonic surgery. She had spiked fevers four times a day in the last 2 days and was severely anemic due to iron deficiency. She received PRBCs with isolated enzyme-phase incompatibility, and during the transfusion, she developed fever, resulting in the premature termination of the transfusion. We evaluated the patient 8 h after the transfusion. The patient denied having suffered from back pain, chest pain, or dark discoloration of the urine. Her hemodynamic status remained stable. Posttransfusion tests were negative for hemolysis. The patient's direct Coombs test was negative. No signs of hemolysis were observed during subsequent daily visits. The patient's fever subsided by day 3. Given the patient's underlying medical condition with frequently rising body temperatures, we attributed the fever to the pelvic abscess rather than a transfusion reaction.

The second patient in Group I was a 22-year-old female in the 18th week of her first pregnancy with a retroperitoneal mass. She required transfusion support due to anemia of chronic inflammation. She was transfused with PRBCs with isolated enzyme-phase incompatibility. Fever and chills were recorded in the first minutes of the transfusion, the infusion was terminated, and blood samples were obtained for further evaluation. The patient reported no back or chest pain and no darkening of the urine. Posttransfusion blood samples did not show signs of hemolysis. Antibody screening was negative. The patient was considered to have experienced a febrile nonhemolytic transfusion reaction. She was later diagnosed with tuberculosis, received appropriate treatment, and delivered a healthy infant.

The third patient in Group I was a 28-year-old female carrying triplets in the 27th week of gestation. She was referred to the emergency department with the diagnosis of placental abruption. All three fetuses were found dead. She demonstrated hemodynamic compromise and disseminated intravascular coagulation. Emergency cesarean delivery was performed. Postoperatively, she was found to be anemic, primarily due to blood loss. She was transfused with one unit of PRBCs with isolated enzyme-phase incompatibility after several crossmatching attempts failed to identify a fully compatible product. Late during the transfusion, the patient complained of

feeling claustrophobic and demanded the infusion be stopped. The attending physician described this as psychological distress and noncompliance in his report rather than a transfusion reaction. We visited the patient the next day and found no symptoms or signs consistent with a transfusion reaction. Her lactate dehydrogenase (LDH) level was elevated; however, a comparison with the level before the transfusion was unavailable. The blood samples obtained after the transfusion were centrifuged and the serum component was bright yellow. The haptoglobin level was within normal limits. The elevated LDH level was considered to be associated with dead-fetus syndrome and the catabolic changes leading to the obstetric emergency. The patient uneventfully received two units of PRBCs the next day. She was discharged 3 days after the surgery.