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Whole blood transfusion in common marmosets: a clinical evaluation

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Abstract: In veterinary medicine, blood transfusion is commonly performed on companion animals. The common marmoset is a small nonhuman primate with increasing popularity as an animal model in biomedical research. Because of its small whole blood volume, the marmoset is at high risk of exsanguination, and blood transfusion is required to care for life-threatening bleeding. However, few clinical evaluations exist on transfusions for marmosets. This study performed whole blood transfusion with cross-matching on nine marmosets and surveyed the therapeutic effects. Recipients included clinical cases with persistent bleeding, anemia, and coma, as well as animals subjected to postoperative bleeding prophylaxis. Donors were selected from healthy marmosets, including littermates. Cross-match assay before transfusion were all negative, and recipients showed no visible signs of transfusion-related adverse reactions. Whole blood transfusions caused hemostasis and successful recovery in bleeding marmosets, including long-term improvement of anemia cases. Our results indicated that blood transfusion is effective for marmosets with severe anemia and persistent hemorrhage from both non-experimental and surgical causes. Furthermore, DNA sequencing for blood-group classification revealed that all subject marmosets were type A, suggesting that the risk of blood type mismatch may be low in this species.

Key words: blood, blood transfusion, common marmoset, cross-matching, veterinary medicine

Introduction

The common marmoset (marmoset, *Callithrix jacchus*) is a New World primate species frequently used for biomedical research and preclinical studies because it is closely related to humans, small (300–500 g), and reproduces rapidly for a primate [1]. Recent advances in genetic modification technologies have expanded the species' research usability as a translational nonhuman primate model [2–5]. Many of these new research techniques require surgical treatments for analyzing physiological status, such as two-photon calcium imaging [6], multielectrode arrays [7], or awake MRI with attached

head post [8]. Sophisticated veterinary management techniques are indispensable for performing these advanced analyses while maintaining marmoset well-being [4, 9, 10].

Due to their small size, marmosets have a relatively high risk of fatal blood loss. An adult of average weight (350 g) has an estimated circulating blood volume of 24.5 ml, meaning it can only lose 4.9 ml before reaching the acute bleeding volume that causes hemorrhagic shock (20% circulating blood volume) [11]. In veterinary medicine, including of non-human primates, blood transfusion is common and generally applied to anemia, hemorrhage [12], hemolysis, or ineffective erythropoi-

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Supplementary Figure and Tables: refer to J-STAGE: <https://www.jstage.jst.go.jp/browse/expanim>



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esis to increase oxygen-carrying capacity [9–14]. However, no detailed research on the clinical effects and safety of blood transfusions has been reported for the marmoset.

Therefore, this study performed whole blood transfusion on nine marmoset cases. We included a cross-matching assay to avoid adverse reactions. We then surveyed evaluated the therapeutic and negative effects of transfusion.

Materials and Methods

Animal facilities

This study was conducted at the animal facility in the Central Institute for Experimental Animals (CIEA), Kawasaki, Japan. The experimental protocol was approved (approval number 17051) by the Institutional Animal Care and Use Committee, according to CIEA Regulations for Animal Experiments, based on Guidelines for the Proper Conduct of Animal Experiments from the Science Council of Japan (2006). Marmosets were kept in cages with family (820 × 610 × 1,578 mm), in pairs (409–820 × 610 × 728–1,578 mm), or alone (409–820 × 610 × 728–1,578 mm) depending on experimental and veterinary care reasons. Cages were positioned facing each other, allowing subjects to communicate visually and vocally. Each cage is equipped with a sleeping area, wooden perches, and hammocks for environmental enrichment. The animal rooms were conditioned at 26–28°C and 40–60% humidity with a 12:12 h light/dark cycle. Animals were negative for *Salmonella* spp., *Shigella* spp., and *Yersinia pseudotuberculosis* in yearly fecal examinations.

Blood-transfusion recipients

Recipients were 9 marmosets (5 females and 4 males, R1–9) aged 2.3–9.3 years (Table 1). Their weights ranged from 315–432 g at the time of transfusion. Before trans-

fusion, 0.2–0.3 ml of blood was collected from the femoral vein and used for blood tests, except in a fatal case (R1). In R1, 0.1 ml of blood was collected only for the hematocrit test with a capillary tube and cross-matching assay. A complete blood count (CBC) was performed for all cases using Sysmex XT-2000i (Sysmex Corp., Kobe, Japan), except for R1. Blood biochemical tests were performed using DRI-CHEM 7000 (Fujifilm Corp., Tokyo, Japan), depending on the amount of plasma that could be collected.

Reference CBC values were acquired using 46 two-year-old marmosets (22 females, 24 males) kept at CIEA. These individuals, had no history of experimental use and no outward health abnormalities. Blood samples were collected from the femoral vein without anesthesia and tested using Sysmex XT-2000iV. Mean ± 2SD of each value was calculated as reference range.

Donor selection

Donors were 7 animals (2 females and 5 males, D1–7) aged 2.8–9.3 years and with a body weight of at least 310 g (Table 1). Animals that had not donated blood within the past month were selected as donors. D1 and D5 donated twice in this study after 27- and 9-week intervals, respectively. Three donor animals were littermates of their recipients (D2, 3, 6). The remainder were not related to their recipients within the second degree of consanguinity. To minimize adverse reactions, recipient littermates were prioritized as donors because their hematopoietic chimerism confers immunotolerance [15–17]. Prior to transfusion, 0.3–0.4 ml of blood was sampled from the femoral vein of candidate donors. These were used for cross-matching and CBC.

Cross-matching assay

The standardized tube test was used to perform major (donor red blood cells [RBC] and recipient plasma) and minor (donor plasma and recipient RBCs) cross-match-

Table 1. The pairs of recipients and donors for blood transfusion and results of cross-match assays

Recipients				Donors					Cross match assay		
No.	Sex/Age ^a	ABO genotyping	BW ^b (g)	No.	Sex/Age ^a	ABO genotyping	BW ^b (g)	Relationships between recipients	Blood volume (ml)	Pre ^c	Post ^d
R1	M/3.2 y	A	301	D1 ^e	F/4.5 y	A	491	Unrelated	2	Negative	Negative
R2	F/8.5 y	A	295	D1 ^e	F/5.0 y	A	443	Unrelated	2	Negative	Negative
R3	F/3.3 y	A	310	D2	M/3.3 y	A	318	Littermate	2	Negative	Negative
R4	M/8.6 y	A	330	D3	M/9.3 y	A	342	Littermate	2	Negative	Negative
R5	M/9.3 y	A	373	D4	F/5.4 y	-	442	Unrelated	2	Negative	Negative
R6	M/7.0 y	A	327	D5 ^e	M/2.8 y	A	420	Unrelated	2	Negative	Negative
R7	F/2.3 y	A	437	D5 ^e	M/3.0 y	A	361	Unrelated	1.7	Negative	Negative
R8	F/8.3 y	A	410	D6	M/8.3 y	A	435	Littermate	2	Negative	Negative
R9	F/6.2 y	A	401	D7	M/8.9 y	A	413	Unrelated	2	Negative	Negative

^aAge (in years) at transfusion, ^bBW: body weight, ^cPre: just before transfusion, ^dPost: 8–41 weeks after transfusion, ^eUsed twice as donors.

ing, as previously described [18]. Briefly, heparin-anticoagulated recipient and donor blood were centrifuged for 15 min at $1,000 \times g$ and 4°C to collect plasma, which was stored in a 1.5 ml tube. Next, RBCs were washed with 1 ml of physiological saline and centrifuged for 15 min at $1,000 \times g$ and 4°C . After washing two to three times with physiological saline, 1–2% RBC saline suspension was prepared. This suspension was mixed with plasma ($20 \mu\text{l}$ each) in a 1.5 ml tube and left standing for 30 min at room temperature. A drop of the mixture was placed on a slide and coverslipped for microscopic observation of RBC aggregations. The suspension and plasma (donor-recipient and recipient-donor) were both tested. Self-combinations were negative controls.

Blood transfusions and follow-up

After confirming negative cross-match results, whole blood transfusions were performed. Blood (1.7–2 ml) was taken from the femoral vein of each donor and immediately mixed with Citrate-Phosphate-Dextrose-Adenine (CPDA-1) solution (Terumo Corp., Tokyo, Japan) to a final concentration of 0.15% for anticoagulation. For case R1, heparin sodium (Mochida Pharmaceutical, Tokyo, Japan) with a final concentration of 1.2 U/ml was used instead. In following the good practice guidelines of the European Center for the Validation of Alternative Methods and the European Federation of Pharmaceutical Industries and Associations, the amount of blood drawn was kept below 15% of the circulating blood volume. The maximum volume of blood collected during this study was 2 ml, which is equivalent to 400 ml in humans.

All transfusions were performed in veterinary intensive care units (ICU, Dear M, Fukuda ME Kogyo, Tokyo, Japan) maintained at $30\text{--}34^{\circ}\text{C}$, 40% humidity, and 25–35% oxygen (Fig. 1). Before transfusion, a recipient animal was sedated via intramuscular injection of a solution containing 0.04 mg/kg medetomidine (Domitor, Kyoritsu Seiyaku, Tokyo, Japan), 0.4 mg/kg midazolam (Dormicum, Astellas Pharma, Tokyo, Japan), and 0.5 mg/kg butorphanol (Vetorphale, Meiji Seika Pharma, Tokyo, Japan) (R5). Two recipients were administered of 0.15 mg/kg midazolam and 0.15 mg/kg butorphanol (R6, 7). Recipients (R2, 4) under no anesthesia were held in a restrainer (CL-4532; CLEA Japan, Tokyo, Japan) when necessary.

Blood was transfused through intravenous injection of the tail vein secured with an indwelling catheter (24G, Top Corp., Tokyo, Japan), butterfly needle (21G, Top Corp.), and PRN adapter (BD, Franklin Lakes, NJ, USA). Flow rate was controlled with a syringe pump (KDS100, KD Scientific, Holliston, MA, USA) (Fig. 1). The flow rate was set at 3 ml/kg/h until 15 min from the start of transfusion, then decreased to 1 ml/kg/h for the first 3 min before increasing to 5 ml/kg/h. This rate was maintained until transfusion was complete, and then up to 8 ml of saline was injected at the same flow rate to flush the line. From the start of transfusion to 60 min after the end, recipients were carefully monitored for adverse reactions, including high fever, shock, and anaphylaxis. They were followed for a year after transfusion to record monthly body weights and clinical incidents. Re-blood tests and re-cross-match assays between the same donor/

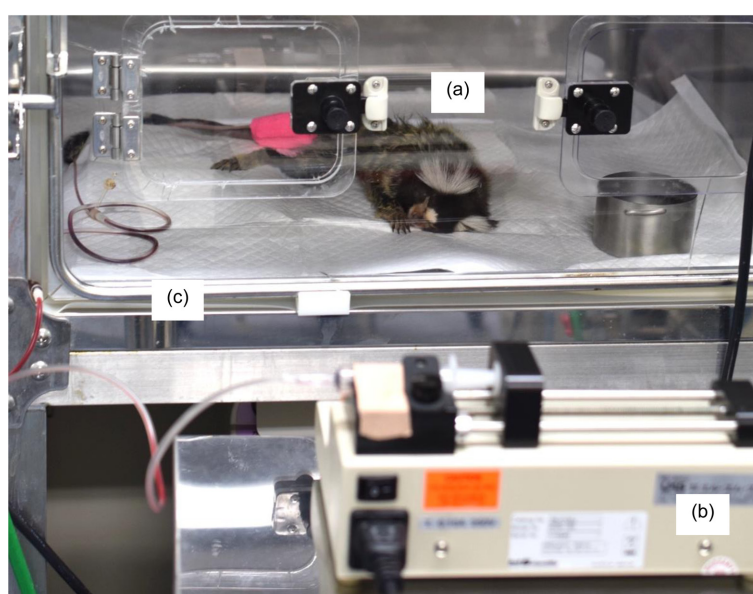


Fig. 1. Recipient marmoset. (a) Individuals received whole blood transfusion using syringe pump (b) in an animal ICU unit (c). Donated blood was administered through the tail vein.

recipient pairs were performed at 8–4 weeks post-transfusion.

DNA sequencing for blood types

To investigate the risk of transfusion due to blood group mismatch, the ABO blood group of the marmosets was tested after transfusion. All recipients, 6 donors (D1–3, D5–7), and 3 other marmosets (1 male, 2 females aged 0, 5.1, and 8.7 years old) were tested. D4 was transported out of the facility immediately after transfusion, so its sample could not be obtained.

Genomic DNA was extracted from hair roots of upper arms or skin of the thigh using the phenol-chloroform-isoamyl alcohol method [19]. A partial region (321 bp) in exon 7 of the *ABO* gene was PCR-amplified using existing primers (forward: TCAGCGACTTCTGC-GAGCGG, reverse: GGCCAATGGCATCGAGGCCG). This region contains a single nucleotide polymorphism that distinguishes blood types. The reaction volume (20 μ l) was prepared with KOD One enzyme solution (Toyobo, Osaka, Japan), according to manufacturer protocol. The thermocycling schedule was as follows: 94°C for 2 min, 5 cycles of 98°C for 10 s and 74°C for 30 s, 5 cycles of 98°C for 10 s and 72°C for 30 s, 5 cycles of 98°C for 10 s and 70°C for 30 s, 25 cycles of 98°C for 10 s and 68°C for 30 s, and finally 68°C for 5 min. Purified amplicons (1/20th of reaction volume) were directly sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA) and the 3730xl DNA Analyzer (Thermo Fisher Scientific).

Statistical analysis

A paired Student's t-test was used to compare CBC values of recipients pre- and post-transfusion. Statistical analyses were performed in R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Cross-match assays

Cross-matching took approximately 60 min per donor-recipient pair. Before transfusion, neither major nor minor cross-matches of any pair showed RBC agglutination (Table 1). Re-cross match assays were negative in all animals at 8–4 weeks post-transfusion (Table 1).

Blood transfusions

Recipients did not show visible signs of transfusion-related adverse reactions. Six recipients had non-experimental persistent gingival (R1–6) or vaginal (R2) hemorrhage, despite of 2–5 days of primary treatments such as pressing, cauterization, and administering hemostatic agents (e.g., tranexamic acid, Table 2). Five out of 6 recipients exhibited hypothermia (<36.5°C), 3 were comatose, 2 had low activity, 1 had melena, and 1 was vomiting (Table 2). Blood examinations revealed severe anemia with hematocrit values of 10–22.4%, hypokalemia (<3.5 mEq/l) (3 recipients), hypercreatininemia (>0.4 mg/dl) (2), high blood urea nitrogen (BUN; 25.6 mg/dl) (3), and high alanine aminotransferase (ALT; 14 IU/l) (1) (Table 2 and Supplementary Table 1) [1]. After transfusions, any hemorrhage was not observed in 5 recipients (R1–4, R6). The 3 comatose cases (R1–3) and 2 low-activity cases (R4 and R6) recovered by the completion of transfusion (Supplementary Fig. 1). Case R5 presented mild gingival re-bleeding at 1 day post-transfusion, which was stopped through stanching with a cauterizing knife.

Three recipients (R7–9) were transfused to prevent postoperative bleeding from ovum pick up (OPU) (R7) and cesarean sections (R8–9) [3, 20]. Case R7 presented no obvious preoperative clinical abnormalities. Although we observed intra-abdominal hemorrhage after OPU surgery, we could not accurately identify the bleeding

Table 2. Clinical characteristics of blood transfusion recipients

No.	Category	Bleeding sites	Periods of primary treatments	Ht (%)	Other signs/remarks
R1		Gingiva	3 days	10*	Anemia, comatose, hypothermia, melena
R2		Gingiva, vagina	2 days	16.8	Anemia, comatose, hypothermia
R3		Gingiva (around first premolar)	5 days	13.0	Anemia, comatose, hypothermia, K \downarrow
R4	Clinical cases	Gingiva	2 days	12.6	Anemia, low activity, hypothermia, BUN \uparrow , Cre \uparrow , K \downarrow
R5		Gingiva (around mandibular canine)	2 days	46.2	Re-bleeding after transfusion, ALT \uparrow
R6		Tongue	2 days	22.4	Anemia, low activity, hypothermia, vomiting, BUN \uparrow , Cre \uparrow , K \downarrow
R7		Unidentifiable (abdomen)	Unknown	44.5	Post-surgery intra-abdominal hemorrhage, BUN \uparrow
R8	Experiment-related cases	Surgical wound after surgery	3 hours	35.0	Cesarean section, bleeding from surgical wound
R9		No bleeding (preventive transfusion after surgery)	Not applicable	43.8	Cesarean section

Ht: hematocrit, K \downarrow : hypokalemia (<3.5 mEq/l), BUN \uparrow : high blood urea nitrogen (>25.6 mg/dl), Cre \uparrow : hypercreatininemia (>0.4 mg/dl), ALT \uparrow : high alanine aminotransferase (>14.0 IU/l), *Measurement by capillary tube.

site. For this subject, blood transfusion was performed after the operation to achieve hemostasis. Even though R8 presented no major abnormalities before or during surgery, it did not stop bleeding from the surgical wound post-operation, and blood transfusion became necessary for hemostasis. R9 presented no major bleeding during the perioperative period, but blood transfusion was performed to prevent weakness. All 3 surgical cases recovered with no visible hemorrhage after transfusion. The mothers (R8 and R9) were able to exhibit parental behaviors at 1 and 7 days later, respectively.

Post-transfusion follow-up

Donors were in good physical condition after transfusion. They were clinically followed for 3 months and then returned to experimental use. Recipients showed no transfusion-related disorders during the 1 year follow-up period. Except for R8, none exhibited notable post-transfusion weight loss (Fig. 2). However, 2 cases were euthanized during the follow-up period. The aforementioned R8 was euthanized 18 weeks post-transfusion due to severe weakness associated with weight loss and mild anemia, diagnosed as marmoset wasting syndrome (MWS) [21, 22]. Additionally, R1 was euthanized 4 weeks post-transfusion for acute collapse; the necropsy revealed abdominal blood coagulation adhered to the mesentery.

During 8–4 weeks post-transfusion, CBC tests revealed that all anemia cases recovered; hematocrit (HCT), red blood cell count (RBC), and hemoglobin (HGB) significantly increased post-transfusion ($P<0.05$) (Figs. 3A–C). Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) ranged around reference values at pre- and post-transfusion (Figs. 3D and E). One exception was R2, with higher MCV and MCH than reference values after transfusion. R3 was slightly lower than reference values for mean corpuscular hemoglobin concentration (MCHC) after transfusion (Figs. 3D–F). White blood cell count (WBC) significantly decreased after transfusion ($P<0.05$), but all cases were within the reference value (Fig. 3G). Platelet count (PLT) was within the reference value in all recipients before transfusion, but after transfusion, R2 values were highly elevated (Fig. 3H). Reticulocyte ratio (RET (%)) in three cases with severe pre-transfusion anemia (R2–4) decreased to the reference range post-transfusion (Fig. 3I).

DNA sequencing of ABO blood groups

Nucleotide sequences (280 bp) were identical across all 18 samples and matched with known marmoset *ABO* sequences. The SNP sequence was CTGgggGGG and presumed to be type A (Supplementary Table 2).

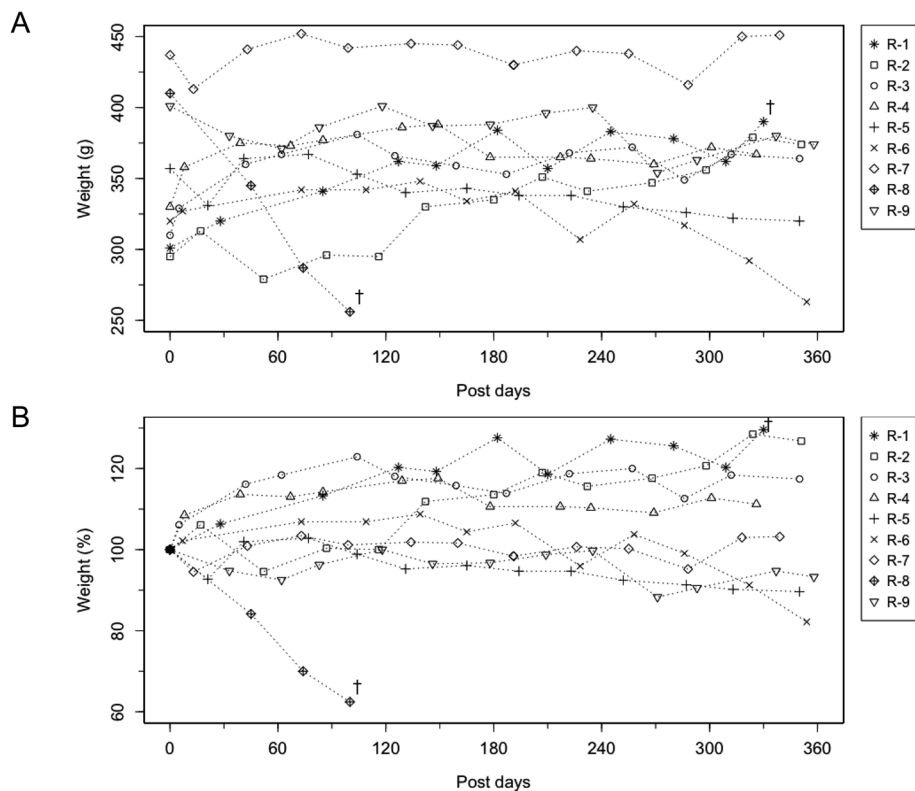


Fig. 2. Bodyweight changes in the recipients for one year post transfusion period. (A) Bodyweight (g). (B) Percent weight change (%). †R-1 and 8 were dead at these time points.

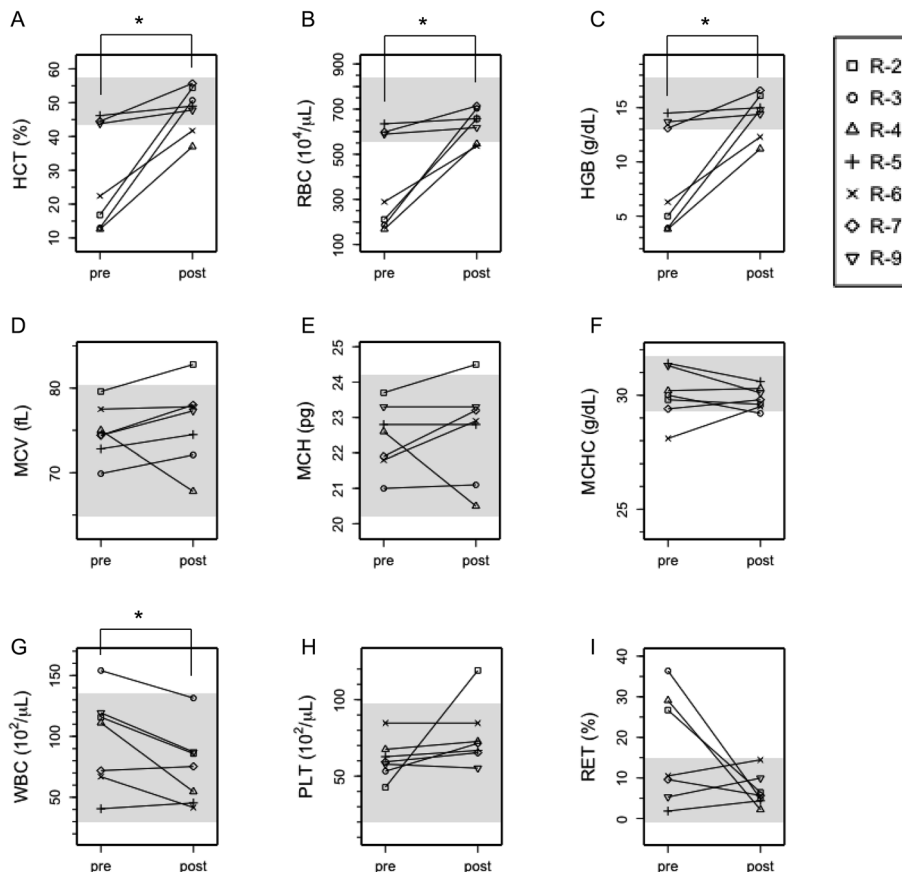


Fig. 3. Complete blood cell count (CBC) in recipients pre- and post-transfusion. We recorded CBC just before transfusion (pre) and 2–8 months after transfusion (post). * $P < 0.05$ (paired *t*-test). (A) HCT: hematocrit, (B) RBC: red blood cell, (C) HGB: hemoglobin, (D) MCV: mean corpuscular volume, (E) MCH: mean corpuscular hemoglobin, (F) MCHC: mean corpuscular hemoglobin concentration, (G) WBC: white blood cell, (H) PLT: platelet, (I) RET: reticulocyte. The reference value is indicated by gray shading.

Discussion

In this study, we successfully performed whole blood transfusions in 9 marmosets. Immediately and for weeks after the procedure, recipients recovered from state of debility and stopped bleeding, without any noticeable incidents on either them or donors. R1–6 were clinical cases that were mainly due to spontaneous oral bleeding, which we suspect to have been caused by trauma. There were no obvious underlying diseases that could have caused continuous bleeding. R4 and R6 were cases that had traumatic hemorrhage as etiology since the bleeding was caused by obvious trauma in the mouth, although blood tests revealed that the renal function might have been impaired. In these cases of traumatic bleeding, hemostatic agents were administered until just before transfusion; however, there was no response. The hemostatic effect was observed only after transfusion. In our past experience, marmosets in a coma due to severe blood loss were euthanized because of humane endpoints. In the present study, the fatal cases were rescued

by blood transfusion, which can contribute to reduce mortality in captive marmosets.

R7–9 were experiment-related cases that were transfused for bleeding associated with surgery. The underlying diseases of R7–9 are unknown. In the case of bleeding from the surgical wound (R8), the bleeding did not respond to hemostatic agents but stopped immediately after transfusion. In R7 and R9, no bleeding was observed after transfusion, and the animals were in good condition. These results suggest that blood transfusion can contribute to the improvement of animal conditions even in surgical procedures associated with bleeding. The overall positive outcome could be attributed to the hemostatic effect of whole blood transfusion, which replenishes coagulation components and platelets in plasma [23].

Our post-transfusion follow-ups (Figs. 2 and 3) revealed long-term improvements in anemia for 4 animals, with good prognosis in most cases. Case R2 showed abnormal values in post-transfusion CBC tests at 18 weeks (Fig. 3), but by 54 weeks, all parameters were

within the reference range for MCV, MCH, and PLT (Supplementary Table 3). The two euthanized animals suffered from MWS (R8) and intra-abdominal bleeding (R1), both apparently unrelated to blood transfusion. R1 was not used in any prior experiment and the cause of bleeding was unknown. The results of CBC tests at 47 weeks post-transfusion indicated that intra-abdominal bleeding began a week before euthanasia (Supplementary Table 3).

Our findings suggest that the blood transfusion method we used is safe for both recipients and donors. Donated blood volume was 1.7–2 ml per animal, lower than the acceptable sampling volume of <15% body weight [11]. Immobilization with a restrainer or sedatives is necessary for safe transfusion because marmosets have high motor ability and finger dexterity. For this purpose, combinations of butorphanol, an analgesic opioid, could be a good sedative option to relieve pain in diseased animals.

Pre-transfusion blood cross-matching is recommended for both humans and companion animals to prevent adverse effects such as shock, high fever, hemolysis, and coagulation [13, 24]. However, previous marmoset studies rarely describe blood cross-match assays [9, 10]. In this study, the results of cross-matching were negative in all pairs (Table 1). Specifically, we examined ABO blood groups because they are the critical determinant of transfusion incompatibility in humans; blood type is determined from allelic variation at the *ABO* gene, which encodes a glycosyltransferase [25, 26]. We found only type A among our marmoset samples, corroborating previous reports at three marmoset facilities that the primates were all type A [26]. Furthermore, re-cross matches after transfusion were negative in every pair. These results imply that marmosets are less likely to have primary and secondary immune responses to transfused blood. Therefore, they may be able to have multiple blood transfusions more safely than other species. In other non-human primates, some non-ABO antigens have been reported using commercial microtyping cards [27]. Kell, Lutheran, Kidd, and Duffy antigens were detected in rhesus macaques, baboons, and squirrel monkeys. Rh antigens were detected in squirrel monkeys. Since marmosets were not examined in the exhaustive study, it is possible that other blood group systems exist in marmosets as well. Because of this possibility, there is a need to perform a pre-transfusion cross-match assay for safe transfusion even in marmosets, where only type A has been reported. For urgent cases that cannot wait for cross-matching results, using littermates as donors is theoretically acceptable because marmoset littermates are known to be allo-tolerant because of hematopoietic

chimerism [28].

Transfusion of whole blood containing active white blood cells poses a risk of graft-versus-host disease (GVHD) [29]. Since no blood preservation method has been established for marmosets, transfusions were performed using blood collected just before transfusion. Nine patients did not experience any post-transfusion side effects or thrombocytopenia. Therefore, it is believed that GVHD did not occur. In the future, it will be necessary to study the method of leukocyte removal and appropriate radiation exposure for a safer transfusion method.

In one animal, we performed preventive transfusion immediately after cesarean section. Obstetric hemorrhage remains an important cause of maternal morbidity in humans, and blood transfusions were performed in 0.5% of pregnant women during cesarean sections [30]. Thus, we felt that preparation for a preventive blood transfusion may be necessary before cesarean section in marmosets.

In conclusion, we showed a practical technique for cross-match assay and whole blood transfusion in marmosets. Our results indicated that blood transfusion is very effective for marmosets with severe anemia and persistent hemorrhage from non-experimental and surgical causes, which was the first report of practical cases in this species. Furthermore, there was no deterioration in the recipients' condition that could be associated with blood transfusion. Safe blood transfusion should contribute to improving the welfare of marmosets in biomedical research.

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