







Effects of Increased Storage Period of Erythrocyte Suspensions on Patient Outcomes in Pediatric Patient Population Undergoing Craniosynostosis Surgery

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ABSTRACT

Objective: Although the blood transfusion required due to bleeding caused by the large incision area during craniosynostosis surgery is necessary to maintain the oxygen-carrying capacity of the blood, it carries significant risks. Our aim in this study is to determine the effect of the storage period of erythrocyte suspensions (ESs) on patient outcomes in the postoperative period in a pediatric patient group who underwent craniosynostosis surgery.

Methods: Fifty-six patients aged 2-24 months who had undergone craniosynostosis surgery between 2018 and 2021 were included in our retrospective study. The patients were divided into two groups according to whether the storage time of the given ES and fresh frozen plasma (FFP) (time elapsed from the day it was received from the donor to the day it was transfused into the patients) was less or more than 10 days. Demographic data of the patients, mean arterial blood pressure, body temperature, hemoglobin (Hb), INR, platelet (plt) values, intraoperative bleeding, amount of transfusion, amount of crystalloid and colloid fluid administered, duration of operation, duration of anesthesia, complications, duration of stay in the postoperative intensive care unit (ICU), amount of transfusion given by drainage, and length of hospital stay were recorded.

Results: The mean age of the patients was 8.27 ± 6.294 months. A positive correlation was found between the storage time of ESs used in the intraoperative period, ES and crystalloid amount, and postoperative drainage. A negative correlation was found between intraoperative FFP transfusion and the change in Hb values measured in the ICU, and between intraoperative ES transfusion and plt values on ICU discharge ($p < 0.05$).

Conclusion: Our study concluded that the shortening of the storage time of ESs and FFPs may have a positive effect on postoperative drainage. However, since it was found to have no effect on hospital and ICU stay, it is thought that larger-scale studies are needed.

INTRODUCTION

Although blood transfusion is necessary to maintain the oxygen-carrying capacity of the blood, this benefit must be balanced with the risks of transfusion. Although this varies for each patient and type of surgery, even small amounts of blood loss result in hypotension in the pediatric patient group and adversely affect oxygen delivery, especially be-

cause the intravascular volume of patients in the pediatric age group is smaller than that of adults and the compensatory response to blood loss is insufficient.^[1]

Craniosynostosis is a condition in which one or more fibrous sutures in the skull ossify prematurely, changing the shape of the skull. Since the skull cannot expand perpendicular to the fused suture, it grows more in parallel with

the closed sutures to compensate. Sometimes the resulting growth pattern can provide the necessary space for the growing brain, but it may result in an abnormal head shape and abnormal facial features, and large area resection and reshaping operations may be required. Massive transfusion is a common practice during craniostylosis surgery due to the tissue damage that this large incision area causes.^[2] Therefore, in this patient group, which is already susceptible to massive transfusion, correct blood transfusion management is of greater importance, considering the hemoglobin concentration in the blood and the presence of a serious systemic comorbidity in the patient. However, today, in addition to the optimal transfusion policy, the content of blood and blood products, which varies depending on the storage period, emerges as an important factor for the side effects and complications that may develop due to transfusion.

It has been revealed that a series of biochemical and cellular changes occur in erythrocytes during storage. Damage developing in stored erythrocytes, also called storage lesions, poses a risk for tissue perfusion and tissue oxygen delivery. Free hemoglobin released as a result of hemolysis in the bag binds nitric oxide and causes an increase in vascular tone. Free iron can also trigger the formation of reactive oxygen species, causing both kidney or liver damage and cardiac events.^[3] It has been reported that transfusion of erythrocyte suspensions stored in both mice and dogs causes a systemic inflammatory response as a result of increased cytokine release in recipients.^[4,5] In addition, it leads to the growth of ferrophilic bacteria.^[6] During cold storage, oxidative stress and impaired adenosine triphosphate (ATP) metabolism lead to remodeling of the erythrocyte membrane and damage to the cytoplasmic composition.^[7] In addition, loss of cellular antioxidant capacity, concentration changes of K⁺ and Na⁺, loss of membrane and skeletal proteins, loss of membrane lipids, formation of vesicles, and oxidation of skeletal proteins occur.^[8] Furthermore, the stored erythrocytes themselves develop procoagulant activity.^[9] It is well established that stored erythrocyte concentrates are associated with pathological reactions such as immunomodulation and post-transfusion pro-inflammation. The developed microparticles act as pro-inflammatory mediators that induce inflammatory signals as paracrine messengers on the immune system.^[10]

Although all these biochemical and structural changes have been demonstrated, the effect of changes secondary to cellular degeneration, which develops with an increase in the waiting time until the erythrocytes are taken from the donor and transfused, on the clinical outcomes of patients has not been proven in the literature, and clear evidence has not yet been obtained.

Our primary aim in this study is to determine the effect of the retention time of erythrocyte suspension on hospital and intensive care unit (ICU) stay in the pediatric patient group who underwent craniostylosis surgery. Our secondary aim is to reveal its effect on the amount of bleeding that may develop in the postoperative period. Our hy-

pothesis in this study is that erythrocyte suspensions with longer storage times may lead to an increase in morbidity.

MATERIALS AND METHODS

This study was planned retrospectively. Children aged 2-24 months with an ASA score of 1-2, who had undergone craniostylosis surgery at our hospital between 2018 and 2021, were included in the study. Cases with congenital or traumatic extremity pathology, a history of allergy to sensor material, a history of cardiac or vascular disease such as heart failure or hypertension, a body weight of less than 5 kg, those who were admitted to emergency surgery, suffered from surgical complications during the operation, and whose operation lasted longer than 4 hours were excluded from the study.

The data were obtained by reviewing the hospital information system and anesthesia follow-up records of the patients. The storage time of the erythrocyte suspensions given to the patients was recorded by determining the day they were taken from the donor and the day they were transfused to the patients. The obtained data were grouped into two groups. A comparison was made between FFPs and erythrocyte suspensions with a storage period of less than and more than 10 days. Demographic data, including gender, age, and body weight of the patients, were recorded. Arterial blood gas samples were analyzed with the GEN-S hematology analyzer (Beckman-Coulter Inc., Brea, CA). Mean arterial blood pressure, peak heart rate, and body temperature were recorded at the beginning and end of the operation in both groups. Initial and postoperative lactate and pH values were recorded in perioperative arterial blood gas sampling. The amount of intraoperative bleeding; Hb, Htc, INR, and creatinine values at the beginning and end of the operation; the amount of perioperative transfusion of blood and blood products; the amount of crystalloid and colloid fluid administered; the duration of the operation; the duration of anesthesia; urine output; the need for vasopressors; and all the complications that developed in the perioperative period were recorded. The duration of postoperative ICU stay; the amount of transfusion given by drainage; the duration of invasive mechanical ventilation; the need for renal replacement therapy; the Hb, INR, fibrinogen, and creatinine values; the lactate and pH values in blood gas on ICU admission and discharge; reoperation, mortality, cardiac ischemia or infarction that developed during the postoperative period; thromboembolic events; acute respiratory failure; nosocomial infection; acute transfusion reaction; multiple organ failure; and the need for vasopressor or inotropic therapy were recorded.

Statistical Analysis

R version 2.15.3 (R Core Team, 2013) was used for statistical analysis. Descriptive statistical methods (minimum, maximum, median, first quartile, third quartile, mean, standard deviation, frequency, and percentage) were used when reporting the data. Whether the quantitative data

fit the normal distribution was evaluated with the Shapiro-Wilk test and graphical analysis. Independent samples t-test was used in the between-group evaluation of the normally distributed variables. The Mann-Whitney U test was used in the between-group evaluation of the non-normally distributed variables. Analysis of variance and Bonferroni-corrected binary evaluations were used for repeated measures in the within-group evaluations of the normally distributed variables with more than two iterations. Dependent samples t-test was used in the within-group evaluations of the normally distributed variables with two iterations. The Wilcoxon signed-rank test was used in the within-group evaluations of the non-normally distributed variables with two iterations. Pearson chi-square test and Fisher's exact test were used to compare the qualitative variables. Spearman correlation analysis (Spearman's rho) was used to determine the level of correlation between the quantitative variables. Linear regression analysis was used to determine the factors affecting the length of ICU and hospital stay and duration of use of vasopressor agents. Kaplan-Meier plot and log-rank test were used to compare survival between the groups. A P value of <0.05 was considered statistically significant.

RESULTS

Twenty-seven males (48.2%) and 29 females (51.8%) participated in our study. No patient was excluded from the study. When the diagnoses of the patients were examined, 13 patients were diagnosed with brachycephaly (23.2%), 3 patients (5.4%) with multiple suture synostosis, 1 (1.8%) with plagiocephaly and scaphocephaly, 1 with operated lambdoid suture synostosis and brachycephaly, 1 with Apert Syndrome, 1 with Crouzon Syndrome, 1 (1.8%) with calvarial remodeling, 8 (14.3%) with plagiocephaly, 1 with plagiocephaly and scaphocephaly, 12 (21.4%) with scapho-

cephaly, 1 (1.8%) with scaphocephaly and plagiocephaly, and 13 (23.2%) with trigonocephaly. The demographic data of the patients, the values of the blood and blood products used intraoperatively and the perioperative laboratory values, the duration of anesthesia and surgery, and the length of ICU stay are given in Table 1. No significant relationship was found between the age of the red blood cells (RBCs) and fresh frozen plasmas (FFPs) that were used and the intraoperative changes in pH and lactate levels. No relationship was found between the age of RBCs and FFPs used in the ICU and the changes in the Hb, pH, platelet, creatinine, and fibrinogen levels observed during the period between ICU admission and discharge. No relationship was found between the age of the RBCs and FFPs used and the length of ICU and hospital stay. No relationship was found between the age of the RBCs and FFPs used and the amounts of RBC and FFP transfusion performed in the ICU. No significant correlation was found between the age of the RBCs and FFPs used and the amount of colloid and crystalloid fluid administered in the intraoperative period and the amount of intraoperative bleeding. However, it was weakly associated with the amount of postoperative drainage ($r=0.29$, $p<0.05$). There was a very weak positive correlation between the age of RBC used and the amount of urine output ($r=0.30$, $p<0.05$) (Table 2).

The amount of intraoperative RBC and FFP, duration of anesthesia, duration of surgery, and amount of intraoperative colloid and crystalloid were compared with the duration of drainage, ICU, and hospital stay. A significant correlation was found between the amount of intraoperative RBC and crystalloid and the amount of postoperative drainage (Table 3).

There was a very weak significant positive correlation between the postoperative lactate values and the amount of FFP transfused intraoperatively ($r=0.31$, $p<0.05$) (Table 4).

Table 1. Demographic data of the patients, values of intraoperative blood and blood products used and perioperative laboratory values

	Mean	SD	Median	IQR25	IQR75
Month	8.27	6.294	7.00	6.00	9.00
Weight (kg)	8.99	2.309	8.50	7.85	10.00
Storage day of FFP (day)	13.30	8.922	9.00	6.00	22.00
Storage day of ES (day)	10.80	6.423	10.50	6.00	13.75
Intraop bleeding (ml)	227.26	149.356	200.00	145.00	285.00
Intraop ES (ml)	7.75	3.604	7.00	6.00	8.00
Intraop TDP	1.26	0.561	1.20	0.90	1.50
Intraop Hb baseline	9.94	1.039	9.75	9.20	10.67
Intraop Hb end	10.68	1.448	10.55	9.70	11.50
ICU stay (hour)	59.11	58.892	48.00	26.00	74.00
ICU ES tx (ml)	111.16	81.903	120.00	0	150.00
ICU TDP tx (ml)	35.98	69.592	0	0	37.50
Postop drainage (ml)	173.30	90.430	150.00	100.00	217.50
Hospital stay (day)	7.75	3.604	7.00	6.00	8.00

Table 2. The relationship between the age of the RBCs and FFPs used and the amount of colloid, crystalloid given in the intraoperative period, and the amount of intraoperative bleeding and drainage

	FFP (day)		RBC (day)	
	r	p	r	p
Intraoperative bleeding (ml)	0.255	0.140	0.054	0.703
Postoperative drainage (ml)	-0.075	0.659	0.287*	0.032*
Intraoperative bleeding (ml)	0.255	0.140	0.054	0.703
Intraoperative crystalloid (ml)	-0.105	0.536	-0.128	0.345
Intraoperative colloid (ml)	0.217	0.197	0.023	0.869
Anesthesia time (min)	-0.310	0.062	-0.111	0.416
Surgery time (min)	-0.262	0.118	-0.146	0.281

Table 3. The relationship between the amount of intraoperative RBC and FFP, duration of anesthesia, duration of surgery, amount of intraoperative colloid and crystalloid and drainage, ICU stay, and hospital stay

	Postoperative drainage (ml)		ICU stay (hour)		Hospital stay (day)	
	r	p	r	p	r	p
Intraoperative RBC (ml)	0.389**	0.003	0.253	0.062	0.124	0.364
Intraoperative FFP	0.140	0.304	0.045	0.746	0.134	0.326
Duration of anesthesia (min)	0.008	0.954	-0.082	0.553	0.102	0.455
Duration of surgery (min)	-0.009	0.950	-0.055	0.688	0.098	0.473
Intraoperative crystalloid (ml)	0.350**	0.008	0.260	0.055	0.260	0.053
Intraoperative colloid (ml)	0.211	0.119	-0.142	0.300	0.053	0.700

Table 4. Analysis of the relationship between intraoperatively measured lactate values, the amount of RBC and FFP transfused, the duration of anesthesia and surgery, and the amount of crystalloid and colloid fluid administered

	Intraoperative Lactate baseline		Intraoperative Lactate end of surgery		Intraoperative Lactate variance	
	r	p	r	p	r	p
Intraoperative RBC (mL)	0.097	0.475	0.103	0.448	-0.034	0.804
Intraoperative FFP (mL)	0.039	0.775	0.315*	0.018*	-0.235	0.081
Anesthesia time (min)	0.060	0.659	0.054	0.690	-0.013	0.923
Surgery time (min)	0.099	0.466	0.060	0.662	0.003	0.985
Intraoperative crystalloid (ml)	0.097	0.475	0.103	0.448	-0.034	0.804
Intraoperative colloid (ml)	0.053	0.698	-0.026	0.849	0.048	0.725

The difference between the Hb values on ICU admission and discharge and the amount of intraoperative FFP transfusion were very weakly, negatively, and significantly correlated ($r=-0.28$, $p<0.05$). There was a very weak significant positive correlation between the fibrinogen values on ICU admission and intraoperative FFP amounts ($r=0.34$, $p<0.05$). There was a very weak significant negative correlation between the platelet values on ICU admission and intraoperative FFP amounts ($r=-0.32$, $p<0.05$). There was a very weak significant negative correlation between the platelet values on ICU admission and intraoperative RBC amounts ($r=-0.50$, $p<0.001$). There was a very weak significant negative

correlation between the platelet values on ICU discharge and intraoperative RBC amounts ($r=-0.35$, $p<0.001$). The data indicating the effect of FFP age, whether more or less than 10 days, on patient parameters are given in Table 5. The data indicating the effect of RBC age, whether more or less than 10 days, on patient parameters are given in Table 6.

DISCUSSION

Our study investigated the relationship between blood transfusion storage time in pediatric patients undergoing

Table 5. Changes in patient parameters when FFP age is below and above 10 days

FFP day	≤10		10<		p
	Mean	SD	Mean	SD	
ICU ES tx (ml)	107.37	91.414	125.28	88.359	0.549
ICU FFP tx (ml)	63.95	80.287	44.44	80.292	0.465
Postop drainage (ml)	186.84	119.131	165.83	73.008	0.525
ICU creatinin gap	0.0706	0.06682	0.0556	0.10623	0.615
ICU fibrinogen gap	-217.6316	133.71620	-126.8333	85.17197	0.020*
ICU trombosit gap	73.7368	73.55032	54.6111	150.38662	0.623
ICU pH gap	-0.1363	0.09570	-0.1567	0.19629	0.688
ICU lactat gap	1.6368	2.32862	1.5778	1.39603	0.927

Table 6. Changes in patient parameters when RBC age is below and above 10 days

FFP day	≤10		10<		p
	Mean	SD	Mean	SD	
Month	7.54	3.930	9.00	8.009	0.389
Weight (kg)	9.02	1.619	8.95	2.871	0.918
Intraop bleeding (ml)	220.00	170.571	235.40	124.381	0.712
Intraop ES (ML)	-0.1464	0.70579	-0.3214	0.68224	0.350
Intraop FFP (ML)	1.17	0.539	1.35	0.579	0.247
Intraop crystalloid (ml)	525.54	190.114	498.93	185.878	0.599
Intraop colloid (ml)	20.71	34.633	33.93	61.904	0.329
Urine output (ml)	50.18	33.374	81.07	53.253	0.012*
Anesthesia time (min)	264.50	36.943	260.89	39.676	0.726
Surgery time (min)	221.43	36.052	218.57	37.782	0.773
ICU stay (hour)	62.57	77.868	55.52	29.672	0.661
Postop drainage (ml)	162.68	72.194	183.93	105.886	0.384
Hospital stay (day)	7.64	4.262	7.86	2.877	0.826

cranosynostosis surgery and their laboratory parameters and incidence of complications. A positive correlation was found between RBC age and the amount of postoperative drainage. No significant correlation was found between the age of RBCs or FFPs and the length of stay in the hospital or ICU. A positive correlation was found between the amount of FFPs transfused intraoperatively and the postoperative lactate values. No significant correlation was found between whether the age of FFPs or RBCs was more or less than 10 days and the changes in patient parameters.

In the Informing Fresh versus Old Red Cell Management (INFORM) study conducted with a pediatric patient group, no significant difference was found between whether erythrocyte suspension was stored for less than 7 days, 8 to 35 days, or more than 35 days, and the patient outcomes.^[11] In the Age of Red Blood Cells in Premature Infants (ARIPI) study by Fergusson et al.^[12] erythrocyte suspensions stored in the neonatal ICU for less than 7 days and erythrocyte suspensions stored in the ordinary way were compared in 377 premature infants. While the mean

duration was 5 days in the group with less storage time, it was 14.6 days in the group with longer storage time, and morbidity and infection-related results were found to be similar between the two groups.^[12] The results of these studies also confirm our results.

In the study titled Tissue Oxygenation by Transfusion in Severe Anemia with Lactic Acidosis (TOTAL), which investigated the effect of transfusion of erythrocyte suspension with a longer or shorter storage period on blood lactate levels in 290 children (6-60 months) who suffered severe anemia due to malaria or sickle cell disease in Uganda between February 2013 and May 2015, it was found that longer storage time did not lead to higher lactate levels.^[13] Similarly, no correlation was found between FFP age and lactate level in our study. However, the lactate level increased proportionally with the amount of transfused FFP. In light of all this data, it is not possible to reach a conclusion about the effect of the storage period of the blood product on the mortality and morbidity that may develop in patients. The most important feature of this study is that there is no study in the literature that could shed light

on the relationship between the storage time of erythrocyte suspensions used in craniosynostosis operations and patient outcomes in the postoperative period. One of the most important factors affecting patient survival is prolonged hospital or ICU stay. However, no increase was detected in either of them in our study. We believe that this is due to the small sample size.

On the other hand, in a prospective cohort study including 61 trauma patients, a significant correlation was found between elderly blood transfusion and the risk of postoperative infection.^[14] It was argued that this might develop due to the immunomodulatory effects of pending blood transfusion. It was also suggested that the infection could be triggered due to the activation of the neutrophil nicotinamide adenine dinucleotide phosphate oxidase system.^[15] In a study conducted with 128 pediatric patients who underwent palliation of congenital heart disease with cardiopulmonary bypass, longer erythrocyte storage time was associated with a 4-fold increase in postoperative nosocomial infections.^[16] Older erythrocytes may cause a proinflammatory cytokine response, leading to the development of infection. For this reason, the use of leukocyte-reduced blood transfusions has become a current issue.^[17] It was shown that reducing the leukocyte rate in dogs receiving transfusion of blood stored for 28 days does not significantly affect the rate of infection.^[4] Being a controversial issue, leukoreduction was not applied before blood transfusion in our study, and no infectious complication was detected.

Recent publications indicate that acquired fibrinogen deficiency is a leading determinant in the development of perioperative dilutional coagulopathy.^[18] In our study, a significant correlation was observed between the amount of intraoperative RBC and crystalloid and the amount of postoperative drainage. However, no difference was found between the fibrinogen levels, indicating that this may be due to the dilution of other factors. In this case, we believe that positive results will be obtained in clinics by shortening the storage period. In addition, the amount of drainage that would require reoperation was not observed in any of the patients.

Limitations

There are studies advocating that aged erythrocytes are rapidly destroyed after transfusion.^[19] One of the limitations of our study is that the clinical changes and laboratory parameters of the intraoperative period and early postoperative period were investigated. The long-term effects resulting from erythrocyte destruction in the postoperative period were not investigated. Another limitation is that the study design was retrospective, and the sample size was small.

Conclusion

The results of our study concluded that shortening the storage time of blood and blood products may have a positive effect on postoperative drainage. However, larg-

er-scale studies are needed as it was not proven whether this has an effect on hospital and ICU stay and laboratory parameters.

Informed Consent

Retrospective study.

Peer-review

Externally peer-reviewed.

Authorship Contributions

Concept: A.S.; Design: A.S.; Supervision: A.S., T.U.; Fundings: A.S., G.C., G.Ç.; Materials: G.C., G.Ç., C.K.; Data: A.S., G.C., G.Ç., C.K., C.E.; Analysis: A.S., G.C., T.U.; Literature search: A.S.; Writing: A.S.; Critical revision: A.S., G.C., G.Ç., C.K., C.E., T.U.

Conflict of Interest

None declared.

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Kraniyosinostoz Ameliyatı Geçiren Pediatrik Hasta Popülasyonunda Eritrosit Süspansiyonlarının Artan Depolama Süresinin Hasta Sonuçları Üzerindeki Etkileri

Amaç: Kraniosinostoz cerrahisi sırasında geniş insizyon alanının yol açtığı kanama nedeniyle gereken kan transfüzyonu, kanın oksijen taşıma kapasitesinin devamlılığını sağlamak için gerekli olmakla beraber, önemli riskleri bulunmaktadır. Günümüzde kan ve kan ürünlerinin depolama süresiyle değişen içeriğine bağlı gelişen ciddi komplikasyonların postoperatif dönemde hasta sonuçlarını etkileyebileceği de düşünülmektedir. Bu çalışmadaki amacımız, kraniosinostoz cerrahisi geçirmiş olan pediatrik hasta grubunda eritrosit süspansiyonunun depolama süresinin postoperatif dönemde hasta sonuçları üzerine etkisini belirlemektir.

Gereç ve Yöntem: Retrospektif olarak planlanan çalışmamıza, hastane bilgi sistemi ve anestezi izlem kayıtlarından verileri alınarak 2018-2021 yılları arasında kraniosinostoz cerrahisi geçirmiş olan 2-24 aylık 56 hasta alındı. Hastalar verilen eritrosit süspansiyonu (ES) ve taze donmuş plazmanın (TDP) depolama süresine göre (donörden alındığı gün ile hastalara transfüze edildiği gün arası süre) 10 günün altında olan ve 10 günün üzerinde olan şekilde gruplara ayrıldı. Hastaların demografik verileri, ortalama arteryel kan basıncı, vücut sıcaklığı, hemoglobin (Hb), INR, trombosit (PLT) değerleri, intraoperatif kanama, transfüzyonu miktarı, verilen kristalloid ve kolloid sıvı miktarı, operasyon süresi, anestezi süresi, komplikasyonlar, postoperatif yoğun bakımda (YBÜ) kalış süresi, drenaj ile verilen transfüzyon miktarı ve hastanede kalış süresi kaydedildi.

Bulgular: Hastaların ortalama yaşı 8.27±6.294 ay idi. İntraoperatif dönemde kullanılan ES'lerin depolama süresi, ES ve kristalloid miktarı ile postoperatif drenaj arasında pozitif korelasyon saptanmıştır. İntraoperatif TDP transfüzyonu ile YBÜ'de ölçülen Hb değerlerinin değişimi ve intraoperatif ES transfüzyonu ile YBÜ çıkış PLT değerleri arasında negatif yönlü korelasyon saptandı ($p<0.05$).

Sonuç: Çalışmamızın sonuçlarına göre ES ve TDP'lerin depolama süresindeki kısalmanın postoperatif drenaj üzerine olumlu etkisi olabileceği kanısına varıldı. Ancak hastane ve YBÜ'de kalış üzerine bir etkisinin varlığı gösterilememesi daha büyük ölçekli çalışmalara ihtiyaç olduğunu düşündürmüştür.

Anahtar Sözcükler: Eritrosit süspansiyonu; kraniosinostoz; taze donmuş plazma; kan transfüzyonu.