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# Measurement of primary hemostasis potential with platelet function analyzer to investigate the predictive effect on post-operative blood loss in cyanotic and acyanotic pediatric patients

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## ABSTRACT

**Aim:** To investigate hemostatic parameters, including primary hemostasis potential in twenty pediatric patients with or without cyanosis undergoing cardio-pulmonary bypass (CPB) and cardiac surgery to repair congenital defects.

**Methods:** The platelet function analyzer is an instrument that provides a rapid, in vitro, quantitative measurement of platelet adhesion and aggregation in whole blood flowing through a small aperture under high shear conditions. Other parameters monitored included blood loss, prothrombin time, anti-thrombin 3 activity, and fibrinogen and D-dimer levels. Additionally, hematocrit and albumin levels were monitored to assess the level of hemodilution during CPB.

**Results:** Both, cyanotic and acyanotic pediatric patients had evidence of supranormal primary hemostasis potential. Although, measurements in cyanotic patients exhibited a higher percentage ratio, this was found to be statistically insignificant between cyanotic and acyanotic patients (collagen-epinephrine $p$=0.07 and, collagen and adenosine diphosphate (ADP) $p$=0.248). While, in preoperative period, measurements of primary hemostasis potential, coagulation and fibrinolytic system parameters demonstrated no statistically significant difference between cyanotic and acyanotic patients, measurement of prothrombin time, international normalized ratio and thrombin time levels significantly changed in cyanotic patients, after the operation ($p$<0,05). Longer cardio-pulmonary bypass time in cyanotic patients could be responsible for this indifference.

**Conclusion:** The results of our study suggest that the platelet function analyzer system may be an indicator that it can predict bleeding in the postoperative period.

**Key words:** Congenital heart disease, cardio-pulmonary bypass, platelet function analyzer, primary hemostasis.

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## Introduction

Cardiopulmonary Bypass (CBP) is an assistive circulation system used in cardiovascular surgery. One of the main complications due to CBP usage is per-operative hemorrhage [1,6]. Pathophysiology behind these hemorrhages are mainly due to inappropriate activation of coagulation and fibrinolytic systems, contact of coagulation units with artificial surfaces, hemodilution, hypothermia and heparinization of the blood [2,3,6,7].

Platelet Function Analyzer (PFA-100) is a micro-processor system which evaluates primary hemostasis potential in a standardized citrated full blood. It is widely used in operations where hemorrhage risk and primary hemostasis cascade failures are possible, such as; organ transplantation, open heart surgery, orthopedics and interventional cardiology.

In this study, it is aimed to show whether primary hemostatic potential is different in between cyanotic and acyanotic patients, whether PFA-100 has a predictive value over post-operative hemorrhage in pediatric patients who are operated for congenital heart diseases and finally whether there are any significant difference in between cyanotic and acyanotic patients in regards of primary hemostasis potential, coagulation and fibrinolytic system parameters in pre-operative and post-CPB time periods.

## Materials and metods

In this study, effect of CBP on post-operative hemorrhage and blood product transfusion in cyanotic and acyanotic patients is measured by using hemostatic parameters. The study group is divided into two sub-groups; cyanotic and acyanotic. Among these mentioned hemostatic parameters, primary hemostatic potential is measured by using PFA-100 system. Further hemostatic analysis are also made intra-operatively. The changes in coagulation-fibrinolytic systems and primary hemostatic potential are analyzed both quantitative and qualitatively.

Our study group consists of 20 pediatric congenital heart disease patients who are operated in Hacettepe University Cardiovascular and Thoracic Surgery Department. All of the patients are operated with open heart surgery techniques. All patients with hemostatic and systemic disease besides congenital heart diseases and patients who are using any drug affecting coagulation cascade and thrombocyte function are also excluded from the study.

For general anesthesia induction; Pentothal sodium (5mg/kg) and Sevorane (mac=2) or isoflurane (mac=1) were used. Anesthesia is continued with N20-O2 (3lt/min), O2 (3 lit/min), vecunorium bromide (0, 1 mg/kg), phentanyl (0, 1 mg/kg) or morphine sulphate. For antibiotic prophylaxis, cephazolin sodium (50 mg/kg) and gentamicin sulphate (5 mg/kg) are used.

In CBP; hollow fiber membrane oxygenator (D 902 Lilliput 2, Dideco, Italy) and non-pulsatile roller pump (Sarns INC, Ann-Arbor, USA) are used. Prime solution is titrated with ringer lactate solution, the lowest point for hemodulition was determined %20 hematocrit. Furthermore; if lower points were reached, blood transfusion is done according to patient's weight and hematocrit levels.

After midline sternotomy, pericardium is excised and patient is Heparinized (300 IU/Kg) (Nevparin, Mustafa Nevzat) before cannulation, the activated coagulation time (ACT) is titrated to be over 400 seconds, if needed extra doses are administered. After standard aortic-bicaval cannulation, moderate hypothermia is reached (26-30 degrees Celsius) and ice slush is applied. Cardiac arrest is induced with cold potassium

cardioplegia solution (Plegisol Abbot Laboratories, North Chicago, USA). The mean arterial pressure is maintained to be 40-60 mmHg. Neutralization of hepatin is achieved with Protamine Hydrochloride (Protamine 1000, Roche, İstanbul).

Five blood samples are obtained from each patient by using radial and femoral catheter; these obtained blood samples are analyzed within three hours without any delay. For CBC, Vacutainer (Becton Dickinson-France EDTA) tubes are used. Furthermore; for PFA-100 and hemostatic tests citrated Vacutainer tubes are used (Becton Dickinson-France; 3, 8% Na-Citrate). For, biochemical analysis, Diotube (Diomed-Türkiye Silicone) tubes are used.

**Sampling protocol is given below**

- ❖ 1st Sample is obtained immediately after anesthesia induction from arterial catheter.
- ❖ 2nd sample is obtained five minutes before pre-cannulation Heparin administration
- ❖ 3rd sample is obtained, when lowest body temperature is reached.
- ❖ 4th sample is obtained ten minutes after post-CPB, Protamine administration
- ❖ 5th sample is obtained at post-operative 48th hour.

Thrombocyte count is analyzed with CBC by using standard Coulter Counter (STRK, Hialeth, FL). Primary hemostatic potential count is analyzed by using PFA-100 system. (Dade-Behring; Miami, FL, USA). Prothrombin activity, Prothrombin time and INR are analyzed by using sta-Neoplastin CI Plus; activated prothrombin time is analyzed by STA-CK Prest 5i, thrombin time is analyzed by STA-Thrombin, anti-thrombin 3 activity is analyzed by STA-Stachrom AT 3, fibrinogen levels are analyzed by using STA-Fibrinogen 5. Furthermore, white blood cell count, hemoglobin and hematocrit counts are analyzed by standard Coulter Counter (STRK, Hialeth, FL). Albumin levels are calculated by using ALB-plus kit and Modular (Hitachi; Roche) system. Patients' blood drainages and transfusions are calculated during post-operative first day via surgical drainage. No patient in the study group is re-operated due to hemorrhage.

SPSS for Windows (SPSS.Inc) program is used for data analysis. For each studied parameter; mean, standard deviation and standard error values are calculated. To evaluate differences within different phases of operations; tests of within subject effect and paired samples t-tests are used. Furthermore, to assess differences in between cyanotic and acyanotic patients, independent samples t-test is used. For, independent variables, Pearson correlation test is used. $p<0.05$ is considered statistically significant during study.

## Results

When compared to reference values, seventy-five percent of the patients showed higher basal values in pre-operative samples. These values are ninety percent higher on average in cyanotic patients and sixty percent higher on average in acyanotic patients. However, there is no statistically significant difference in between cyanotic and acyanotic patient groups (Table 1).

**PFA-100 system results:**

Collagen-Epinephrine values which is an indicator of Primary Hemostatic Potential (PHP) can be seen in Table 2.

We used tests of within subject effects to assess the difference in between samples gathered during the stages of surgery. According to test of within subject effects analysis, each sample obtained during the stages of surgery has statistically significant difference ($p<0.05$). To assess the difference making stages, we conducted paired samples t-test. In paired samples t-test, each dual group showed statistical

**Table 1.** Descriptive statistical data of the patients in the study group.

| | Age (Year) | Weight (kg) | Height (cm) | CPB time duration (min.) | Amount of Bleeding (ml/kg) | Replacement of | |
|---|---|---|---|---|---|---|---|
| | | | | | | Blood (ml/kg) | Plasma (ml/kg) |
| Cyanotic | 3,7±2,4 | 13,0±4,3 | 92,8±16,3 | 63,5±8,2 | 19,0±13,1 | 5,4±6,3 | 12,4±13,5 |
| Acyanotic | 7,6±4,1 | 22,3±10,4 | 117,2±26,6 | 25,4±11,0 | 8,7±2,5 | 3,4±2,7 | 1,8±2,9 |
| All patients | 5,7±8* | 17,6±9,1* | 105±24,9* | 44,5±21,7* | 13,9±10,6* | 4,4±4,9 | 7,1±11,0* |

*The effect of cyanosis between the groups was evaluated with one-way ANOVA test.*

**Table 2**. Collagen -Epinephrine values.

| Collagen -EPI (87-153 sec.) | 1.Preop | 2. Post Heparin | 3.Hypothermia | 4.Post Protamine | 5.Postop |
|---|---|---|---|---|---|
| Cyanotic | 213±34 | 190±28 | 220±33 | 205±53 | 185±45 |
| Acyanotic | 168±46 | 149±49 | 202±36 | 190±37 | 180±42 |
| All patients | 190±46 | 170±44 | 211±35 | 198±45 | 183±42 |

*Values are given as mean± standard deviation.*

**Table 3.**Collagen -ADP values.

| Collagen -ADP (66-125 sec.) | 1.Preop | 2. Post Heparin | 3.Hypothermia | 4.Post Protamine | 5.Postop |
|---|---|---|---|---|---|
| Cyanotic | 123±23 | 100±19 | 205±34 | 122±38 | 98±19 |
| Acyanotic | 109±27 | 92±20 | 181±29 | 116±35 | 100±15 |
| All patients | 116±25 | 96±20 | 193±33 | 119±36 | 99±17 |

*Values are given as mean± standard deviation.*

significant difference ($p$<0.05). Pearson Correlation Test is used to analyze relationship in between pre-operative Collagen-Epinephrine values and blood loss, blood transfusion and plasma transfusion.

According to Pearson Correlation Analysis Test in paired variables, values below are found.
Collagen-Epinephrine (1st sample) - Blood Loss ($p$<0.05) (r=0.846)
Collagen-Epinephrine (1st sample) - Blood Transfusion (p=0.163) (r=0.478)
Collagen-Epinephrine (1st sample) - Plasma Transfusion ($p$<0.05) (r=0.708)

In these circumstances; there is a statistically significant correlation in between pre-operative Collagen-Epinephrine levels, blood loss and plasma transfusion; however there is no significant correlation in between pre-operative Collagen-Epinephrine and blood transfusion in cyanotic patients.

Pearson Correlation tests is used to assess relation in between pre-operative Collagen-Epinephrine values and blood loss, blood transfusion and plasma transfusion in acyanotic patients.
Collagen-Epinephrine (1st sample) - Blood Loss ($p$<0.05) (r=0.791)
Collagen-Epinephrine (1st sample) - Blood Transfusion ($p$=0.076) (r=0.584)
Collagen-Epinephrine (1st sample) - Plasma Transfusion ($p$=0.606) (r=0.187)

According to Pearson Correlation Test, there is a statistically significant correlation in between pre-operative Collagen-Epinephrine levels and blood loss; however there is no significant correlation in between pre-operative Collagen-

**Table 4.** Platelet counts.

| Platelets (150-450x1000/µl.) | 1.Preop | 2. Post Heparin | 3.Hypothermia | 4.Post Protamine | 5.Postop |
|---|---|---|---|---|---|
| Cyanotic | 217± 87 | 219± 118 | 92±47 | 116±69 | 191± 100 |
| Acyanotic | 234±56 | 236±94 | 102±36 | 110±50 | 153±58 |
| All patients | 225±69 | 227±104 | 97±41 | 113±58 | 172±82 |

*Values are given as mean± standard deviation*

**Table 5.** Thrombin Time test results.

| Thrombin time (15-26 sec) | 1.Preop | 2. Post Heparin | 3.Hypothermia | 4.Post Protamine | 5.Postop |
|---|---|---|---|---|---|
| Cyanotic | 26±18 | Max | Max | 33±11 | 17±1 |
| Acyanotic | 24±12 | Max | Max | 32±11 | 14±1 |
| All patients | 25±15 | Max | Max | 33±11 | 15±1 |

*Values are given mean ± standard deviation.*

**Table 6.** Anti-Thrombin III activity test results.

| AT-3A (%80-120) | 1.Preop | 2. Post Heparin | 3.Hypothermia | 4.Post Protamine | 5.Postop |
|---|---|---|---|---|---|
| Cyanotic | 95±19 | 82±23 | 39±11 | 53±11 | 97±21 |
| Acyanotic | 100±13 | 90±15 | 49±12 | 52±7 | 107±17 |
| All patients | 98±16 | 86±19 | 44±12 | 52±9 | 102±19 |

*Values are given mean ± standard deviation.*

Epinephrine levels, blood transfusion and plasma transfusion. Furthermore; there is no statistically significant correlation in between pre-operative Collagen-Epinephrine levels and thrombocyte count in acyanotic patients ($p$=0.641) (r=0.169).

Collagen-ADP is another parameter is used to show primary hemostatic potential, values of which are shown in Table 3. When compared to reference values, all of the patients showed forty percent higher values on average; furthermore cyanotic patients showed fifty percent whereas acyanotic patients showed thirty percent higher values on average. However, there is no statistically significant difference in samples obtained pre-operatively (1st sample) ($p$=0.278) and post-operatively (5th sample) ($p$=0.248) between acyanotic and cyanotic patients.

Tests of within subject effects is used to show differences in between stages of operations, furthermore there are statistically significant differences in between stages of surgeries in means of Collagen-ADP values ($p$<0.05). To assess the difference making stages, we conducted paired samples t-test. In paired samples t-test, each dual group showed statistical significant difference ($p$<0.05).

Pearson Correlation Analysis Test in paired variables is used to show relation in between Collagen-ADP and blood loss, blood transfusion and plasma transfusion.

Collagen-ADP (1st sample) - Blood Loss ($p$<0.05) (r=0.766)

Collagen-ADP (1st sample) - Blood Transfusion ($p$=0.186) (r=0.455)

Collagen-ADP (1st sample) - Plasma Transfusion ($p$<0.05) (r=0.660)

In these circumstances; there are statistical significant correlations in between pre-operative Collagen-ADP, blood loss and plasma

transfusion; however there is no significant correlation in between pre-operative Collagen-ADP and blood transfusion in cyanotic patients.
Pearson Correlation tests is used to assess relation in between pre-operative Collagen-ADP values and blood loss, blood transfusion and plasma transfusion in acyanotic patients.
Collagen-ADP (1st sample) - Blood Loss ($p$<0.05) (r=0.779)
Collagen-ADP (1st sample) - Blood Transfusion ($p$=0.942) (r=0.026)
Collagen-ADP (1st sample) - Plasma Transfusion ($p$=0.118) (r=0.526)

According to Pearson Correlation Test, in acyanotic patients, there is a statistical significant correlation in between pre-operative Collagen-ADP levels and blood loss; however there are no significant correlations in between pre-operative Collagen-ADP, blood transfusion and plasma transfusion.

**Thrombocyte count**

Thrombocyte count is an important parameter for primary hemostasis; when inspected in the patient group, ninety percent of the patients had physiologic thrombocyte counts. Two patients had mild thrombocytopenia. Thrombocyte counts of the patient group is provided in Table 4.

We used tests of within subject effects to assess the difference in between samples gathered during the stages of surgery. According to test of within subject effects analysis, each sample obtained during the stages of surgery has statistically significant difference ($p$<0.05). To assess the difference making stages, we conducted paired samples t-test. In paired samples t-test, each dual group, except 1st and 2nd group pair, showed statistical significant difference ($p$<0.05) which shows heparinization during operation does not have significant impact on thrombocyte count.

When cyanotic and acyanotic patient groups are inspected, there is no statistically significant difference in pre-operative ($p$=0.312) and post-operative ($p$=0.274) thrombocyte counts. As mentioned before, Collagen-Epi and Collagen-ADP's pre-operative values and thrombocyte counts in the same blood samples had no statistically significant correlation ($p$>0.05).

**Hemodulition**

During CPB; patient's cardiovascular system experiences significant hemodulition. This hemodulition is measured by two separate parameters in our study; hematocrit and albumin concentration.

When hematocrit levels of the patients are inspected, all of the patients had a pre-operative mean hematocrit level of %39, 4 ± 8.3 whereas during CPB, mean hematocrit level decreased to %24.7 ± 6.1. When compared, intraoperative hemodulition resulted in %37 decrease in initial hematocrit levels. Furthermore; when Albumin levels of the patients are inspected, the mean pre-operative (1st blood sample) albumin level was 3.61 ± 0.48 g/dl whereas during CPB, the mean albumin level decreased to 1.65 ± 0.41 g/dl. When compared, intraoperative hemodulition resulted in %54.3 decrease in initial albumin levels.

**Thrombin time**

Thrombin Time (TT) is an easy and fast test to evaluate fibrin formation (Table 5). We used tests of within subject effects to assess the difference in Thrombin Time results in between samples gathered during the stages of surgery. According to test of within subject effects analysis, each sample obtained during the stages of surgery has statistically significant difference (p<0.05). To assess the difference making stages, we conducted paired samples t-test. In paired samples t-test, each dual group, except pair of 1st-4th and 2nd-3rd group pair, showed statistical significant difference ($p$<0.05). TT values of 2nd

and 3rd samples cannot be evaluated due to heparinization of the patients.

The differences in between cyanotic and acyanotic patients groups are determined by using independent t-test. There are no statistically significant difference pre-operatively ($p$=0,766), however in the post-operative period, TT values showed significant differences in between cyanotic and acyanotic patients ($p$<0.05).

Pearson Correlation tests is used to assess relation in between pre- and post- operative TT values and blood loss. In the pre-operative period, there is no statistically significant correlation in between TT values and blood loss ($p$=0,894 r=0,032); however in the post-operative period, TT values and blood loss showed statistically significant correlation ($p$<0.05 r=0,486).

**Anti-thrombin III activity**

Anti-thrombin III (ATIII) is a glycoprotein thrombin inhibitor. ATIII also inhibits, FXIIa, XIa, IXa, Kallikrein and Plasmin. Anti-thrombin activity levels of the patient group can be seen in Table 6.

We used tests of within subject effects to assess the difference in ATIII activity values in between stages of surgery. According to test of within subject effects analysis, each stage of surgery had statistically significant difference ($p$<0.05). To assess the difference making stages, we conducted paired samples t-test. In paired samples t-test, each dual group, except 1st-5th group pair ($p$=0,334), showed statistical significant differences ($p$<0.05). There is no statistically significant difference in between pre-operative and post-operative samples.

Independent samples t-test is used to assess the AT-III activity values differences in between cyanotic and acyanotic patient groups, there is no statistically significant difference in between pre and post-operative AT-III activity values of cyanotic and acyanotic groups ($p$=0,550 pre-op) ($p$=0,295 post-op).

Pearson Correlation test is used to assess the correlation in between pre-operative AT-III, post-operative AT-III activity values and blood loss. There are no statistically significant correlations in between pre-operative AT-III, post-operative AT-III activity values and blood loss ($p$=0.160 r=0,327 pre-op) ($p$=0.069 r=0,414 post-op).

**Post-operative blood loss, blood and plasma transfusion**

As previously mentioned before, average blood loss for cyanotic patient group was 19.0 ± 13.1 ml/kg whereas average blood loss for acyanotic patient group was 8.7 ± 2.5 ml/kg. One-way ANOVA test is used to assess statistical significance of the blood loss difference in between cyanosis status and blood loss, which showed significant difference in between both groups ($p$<0.05).

When the effect of cyanosis on these parameters is tested with one-way ANOVA; the amount of blood transfusions showed no statistically significant correlation ($p$=0,370) whereas blood loss and plasma replacement values showed statistically significant correlations ($p$<0.05).

Pearson correlation test is used to assess the correlation in between Collagen-Epi, Collagen-ADP and blood loss; there is no statistically significant correlation in between these parameters ($p$<0.05 r=0,710 Collagen-EPI, $p$<0.05 r=0,635 Collagen-ADP). Furthermore; Pearson correlation test is also used to assess the correlation in between CBP time, pre-operative hemoglobin levels and blood loss; there are statistically significant correlations found in between these parameters ($p$<0.05 r=0,486 for CPB, $p$<0.05 r=0,656 for Hemoglobin).

## Discussion

One of the most important complications of CPB in both adult and pediatric population is hemorrhages. Re-operation rates due to hemorrhages after CPB used surgeries are in between 1-2% in reported series[8,9]. The pathophysiology behind these hemorrhages are secondary to complex sequence of events such as activation of fibrinolytic systems, contact of blood cells with artificial surfaces, hemodilution, hypothermia and heparinization [2,3,6,7]. Despite the fact that a significant portion of surgeries which requires CPB is performed on pediatric population, a small proportion of publications focus on pediatric population. Furthermore, the current knowledge regarding drug doses preferred in pediatric population is also limited in comparison to adult counterpart [3,4]. In light of these mentioned facts, studies regarding hemostatic dysfunctions in pediatric patients with congenital heart diseases are needed for monitorization of treatment regimens. There is an obvious increased risk of hemorrhage, thrombosis and mortality in pediatric patients with congenital heart diseases, especially in patients with cyanotic heart diseases [10-13]. Furthermore, in patients with cyanosis, previous studies showed fibrinolytic system activations and dysfunctions in coagulation cascades [5,8,10,14]. However, despite these mentioned hemorrhage pathogenesis; there is no laboratory test which would differentiate the patients with high hemorrhage risk [10,15,16].

Thrombocyte dysfunction is thought to be the primary responsible factor in pathogenesis of post-CPB hemorrhage. This dysfunction could be secondary to mechanical stress, contact with artificial surfaces of CPB machine and inappropriate activation of hemostatic and fibrinolytic systems[6,17]. However, a previously conducted study showed number of granulated thrombocytes are significantly lower than degranulated ones [10,18-20]. Furthermore, the mentioned study also includes that inability of counting degranulated granulocytes are secondary to clarification of these thrombocytes from the circulation and fixation of these thrombocytes with white blood cells. After, further evaluation of these thrombocytes under electron microscopy, it was revealed that these cells had undergone through stimuli which resulted in important shape alterations during CPB [21]. Despite the first stimuli in CPB these thrombocytes get desensitized and aggregate, leading to thrombosis and hemorrhages. In our study; it is shown that indicators of primary hemostatic potential, Collagen-Epi and Collagen-ADP, had increased significant during CPB when compared to pre-operative values. However, it is also shown that values of Collagen-Epi and Collagen-ADP returned to pre-operative levels in post-CPB 48th hour.

Ferraris et all. [22] showed in their study that thrombin receptor dysfunction occurs in thrombocytes. Furthermore in the mentioned study, post-operative hemorrhages are thought to be secondary to decrease in response to thrombocyte-thrombin interaction [10,23]. It is also known that relation in between thrombocytes and FXIII depends on thrombin. When previously mentioned factors considered, thrombin regulation is essentially important for hemostasis in pediatric patients with congenital heart diseases. In previously conducted studies, it is shown that CPB increases Thrombin-Antithrombin complex by five-fold. It is believed that during CPB, due to shear stress, production of thrombin increases which is later compensated by anti-thrombin III. Furthermore, it is also believed that FXIII could be a more sensitive marker than thrombocyte granule material for in vivo thrombocyte activation. However, it is also thought that none of the tests mentioned before,

thrombocytes and tests which depends on coagulation and fibrinolysis, cannot predict hemorrhage pre-operatively.

A study conducted by Levin et all. [10] showed that hemodilution results in increased hemorrhages post-operatively due to decreased concentrations of coagulation and fibrinolytic factors. Furthermore in cyanotic patients, it showed that levels of plasminogen activator inhibitor (PAI-1) levels and D-dimer levels are higher than acyanotic patients. In cyanotic patients, the overall operation time is higher than acyanotic groups; also in the cyanotic patient group overall age is lower than acyanotic patients. When all of these previously observations and studies are considered, it could be concluded that cyanotic patients tend to have higher blood loss than their acyanotic counterparts [10]. Levin et al[10] showed in their study that Tranexamic acid application, to decrease post-operative bleeding, had no statistically significant impact on post-operative bleeding. To minimize blood loss and needed transfusion, specific hemostatic disorders should be considered and specific treatment strategies for these patients must be developed. In the previous studies, it is shown that with initiation of CPB, concentrations of factors II, V,VII, IX, XI, XII and pre-kallikrein decreases nearly up to %50 [3]. However; Factor VIII, an acute phase reactant, showed increased concentrations. When compared to other studies in literature; our study applied a similar hemodilution (%24-73) [3,8]. In our study, it was shown that hematocrit levels of the patients showed 37% decrease whereas Albumin levels of the patients showed 54.3% decrease. If blood transfusions done for keeping the hematocrit levels above 20% are included, Albumin levels will provide a more accurate statistical analysis.

In the literature; there are studies stating that there is no statistical significant differences of levels of procoagulant and inhibitor factors in between cyanotic and acyanotic patients [3,8,24]. In our study, we evaluated the results according to the functions of the cascades affected by the same unit in addition to the coagulation unit. It was aimed to show changes where these effected factors are involved in. There are no statistically significant differences of prothrombin time in between cyanotic and acyanotic patient groups.

Furthermore, there are no statistically significant differences of pre-operative aPTT, TT and AT-IIIa values in between cyanotic and acyanotic patient groups. It can be interpreted that there are no significant differences in means of pre-operative coagulative and fibrinolytic system functions in between cyanotic and acyanotic patient groups. Furthermore; there were statistically significan changes for each parameter of the study with initiation of CPB, which is thought to be secondary to hemodilution, hypothermia and conctact of blood unit with artificial surfaces.

Overall pre-operative thrombocyte count was 225± 69 for the study group whereas during CPB, thrombocyte count decreased to 97± 41. However, there was no statistically significant difference in between thrombocyte counts of cyantoic and acyanotic patient groups. In the literature, there are publications stating the decrease in thrombocyte count and increase of degranulation of thrombocytes during CPB. However, despite the high number of publications in this particular interest, there are points to be further acknowledged. During the first minutes of CPB initiation; there is a rapid decrease [21] in thrombocyte counts whereas concentration of alpha-granules and Thromboxane-A2 increase[21,25,26]. There are increases in degranulation of thrombocytes, the level of this reaction is not at an irreversible level and remains at a tolerable one. This cycle of degranulation and increased concentration of

previously mentioned blood factors maybe an indicator of continuing activation of thrombocytes [21,25,27]. However, studies regarding alpha-granules of the thrombocytes show no excretion of these materials directly from thrombocyte [21,28,29] therefore the increase of these granular material could be secondary to lysis of thrombocytes in CPB [30-32]. Further publications regarding post-operative bleeding in post-CPB phase of the treatment state that primary responsible for the bleeding is aggregation dysfunction of the thrombocytes.

In our study, PFA-100 results revealed statistically significant increases in both Collagen-Epi and Collagen-ADP levels during CPB. This dramatic increase indicates thrombocyte consumption, hemodilution, desensitization and decrease in aggregation capacity. Furthermore; pre-operative PFA-100 results of Collagen-Epi and Collagen-ADP showed statistically meaningful correllation with post-operative bleeding volumes. However, there are no statistically significant correlations in between prothrombin time (PT), activated thromboplastin time (Aptt), thrombin time (TT) and anti-thrombin III (AT-IIIa) activity with post-operative bleeding volumes. Our study showed that thrombocyte function is an important factor for post-operative bleeding in pediatric patients with congenital heart diseases. Furthermore; PFA-100 system is capable of providing statistically significant data regarding thrombocyte functions, primary hemostatic potential and show important prognostic information regarding post-operative bleeding volumes. The monitorization of heparin infusion during CPB is important due to danger of either possible thrombosis of the cannulas and microvasculature [3,33,34] or hemorrhages due to over-heparinization [3,35-38].

Although, heparinization during CPB is monitorized by ACT, there are studies showing absence of meaningful correlation in between ACT values and correct heparinization [3,8,39]. Absence of mentioned correlation can be explained by thrombocyte aggregation, hypothermia and hemodilution [3,39,40]. Furthermore; ACT values cannot address whether post-operative bleeding is due to over-heparinization during CPB or CPB related acquired hemostatic dysfunctions. However, absence of correlation in between ACT and heparin concentrations are important for neutralization of heparin with protamine; because over post-CBP protaminization can also result in thrombocyte dysfunctions. Therefore, ACT values cannot be relied on solely for heparinization-protaminization and PFA-100 can provide important data regarding and address possible cause behind intra-operative and post-operative bleeding.

## Conclusions

Post-operative hemorrhage is one of the most common complications of cardiopulmonary bypass. PFA-100 system is a sensitive, applicable and reliable analyzer for detecting blood loss, primary hemostatic disorders and developing new treatment protocols, accurate heparinization for primary hemostatic disorders in pediatric patients with congenital heart disease.

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