Platelet-leukocyte activation and modulation of adhesion receptors in pediatric patients with congenital heart disease undergoing cardiopulmonary bypass

Cardiopulmonary bypass has been shown in adults to activate platelets and leukocytes, lead to the formation of circulating platelet-leukocyte conjugates, and alter adhesive receptors on both cell types. Pediatric patients with congenital heart disease undergoing cardiopulmonary bypass, however, have not been extensively studied and may represent a group at particular clinical risk for bleeding and pulmonary dysfunction. We studied 13 patients with congenital heart disease undergoing operations necessitating bypass, 7 with cyanotic and 6 with noncyanotic congenital heart disease. We determined that (1) the surface density of platelet glycoprotein Ib was significantly lower at baseline and throughout bypass in patients with cyanotic heart disease than in noncyanotic patients; (2) platelet glycoprotein Ib in both cyanotic and noncyanotic congenital heart disease decreased significantly during bypass, with a nadir of 75 % of baseline values; (3) platelets were activated to a high degree, comparable with that seen in adults; (4) mean circulating monocyte-platelet conjugates rose significantly during bypass, increasing from 36 % to 66 % by the end of bypass, whereas neutrophil-platelet conjugates and lymphocyte-platelet conjugates declined; and (5) both monocytes and neutrophils were activated by cardiopulmonary bypass, as assessed by increased surface expression of CD11b and, in the case of monocytes, CD11b expression continued to increase even after termination of bypass. Patients with cyanotic and noncyanotic heart disease did not differ with respect to platelet or leukocyte activation or the formation of platelet-leukocyte conjugates. We conclude that in children with congenital heart disease cardiopulmonary bypass causes loss of platelet adhesion receptors, activation of platelets, formation of platelet-leukocyte conjugates, and leukocyte activation. Cyanotic and noncyanotic patients are qualitatively similarly affected; however, cyanotic patients demonstrate a baseline deficit in the platelet adhesion receptor glycoprotein Ib. These cellular changes may contribute to both the hemostatic and inflammatory complications associated with cardiopulmonary bypass. (J THORAC CARDIOVASC SURG 1994;107:280-8)

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Structural and functional changes in circulating cells occurring in the adult population during cardiopulmonary bypass (CPB) have been described by us and others. Platelets are activated, resulting in release of α -granule contents and expression of a neoantigen, P-selectin (CD62, GMP-140, PADGEM), on the surface of the activated platelets.¹⁻³ In addition, surface expression of the major platelet von Willebrand factor receptor, glycoprotein (GP) Ib, and the fibrinogen receptor GPIIb/IIIa are both decreased during CPB, although other platelet adhesive receptors such as GPIV remain unchanged.⁴⁻⁶ Leukocyte alterations during CPB include release of

neutrophil elastase⁷ and expression of the adhesion receptor CD11b on the surface of monocytes and neutrophils (PMN).⁸ In vitro studies have demonstrated that the appearance of P-selectin on the surface of activated platelets mediates binding to monocytes and PMN,⁹ but not to the majority of lymphocytes.¹⁰⁻¹² We have previously shown that in temporal parallel to platelet activation during CPB the number of circulating monocytes and PMN with bound platelets increases significantly.⁸ Although the role of this in vivo platelet-leukocyte adhesion is not certain, preliminary work suggests that it may modulate the proinflammatory and prothrombotic effects of PMN and monocytes,^{13,14} and thus contribute to the pathophysiologic effects of CPB.

Children with congenital heart disease (CHD) exhibit a host of unique physiologic characteristics that may place them at particular risk for the hemorrhagic and inflammatory complications associated with CPB. Platelet dysfunction, thrombocytopenia, disseminated intravascular coagulation, and decreases in coagulation factors are prevalent in pediatric patients with either noncyanotic or cyanotic CHD.¹⁵⁻¹⁷ This is especially prominent in the cyanotic population.^{18,19} In addition, the relatively large volume of the CPB circuit as compared with the patient's intravascular volume and the often high pump flow rates used may increase the degree of cellular injury associated with pediatric CPB.

Flow cytometry techniques allow the examination of large numbers of individual cells from small amounts of whole blood fixed immediately after blood drawing, permitting the detection of changes in surface adhesive proteins of platelets and leukocytes "frozen in time." We have used these techniques to study the changes in platelets and leukocytes of cyanotic and noncyanotic children with CHD undergoing CPB.

Materials and methods

Antibodies. All monoclonal antibodies (MoAb) were used as purified whole immunoglobulin G. All experiments included irrelevant isotype-specific mouse MoAb as negative controls. The monoclonal antibody 1E3²⁰ is specific for P-selectin. P2²¹ and SZ2²² (AMAC, Inc., Westbrook, Maine) recognize GPIIb/IIIa and GPIb, respectively. Anti-CD45 (HLE, Becton-Dickinson Immunocytometry Systems, San Jose, Calif.) recognizes a CD45 isoform present on PMN, monocytes, and lymphocytes but neither erythroid cells nor platelets.²³ The MoAb D12²⁴ (Leu15; Becton-Dickinson) recognizes CD11b on monocytes and PMN.

Patient studies. After institutional approval by the Human Investigation Committee of the Yale University School of Medicine was obtained, 13 consecutive patients with CHD undergoing elective operations necessitating CPB were studied. They were divided into a cyanotic group ($n = 7$) and a noncyanotic group ($n = 6$) with cyanosis defined as a preoperative room air oxygen saturation of 85% or less. All patients underwent CPB with a Cobe VPCML membrane oxygenator (Cobe

Laboratories, Lakewood, Fla.) at comparable flow rates. Whole blood samples (200 μ l) were taken from the radial artery catheter and immediately fixed in 1% paraformaldehyde. Samples were taken at the following time points: before start of operation, after systemic heparinization before bypass, 10 minutes after start of bypass, at termination of bypass (before protamine administration), and 1 to 2 hours after bypass. Patient samples were studied for (1) platelet surface density of GPIb; (2) platelet activation (P-selectin expression); (3) leukocyte surface expression of CD11b; and (4) percentage of leukocyte-platelet conjugates.

Fluorescence labeling. All whole blood samples were fixed in paraformaldehyde for 60 minutes at 4° C, then washed three times in Tyrode's-HEPES buffer (HEPES 5 mmol/L, NaCl 140 mmol/L, KCl 2.7 mmol/L, dextrose 5.5 mmol/L, NaH₂PO₄ 0.42 mmol/L, and NaHCO₃ 12 mmol/L, pH 7.4) and divided into three aliquots for study: one for platelet receptors, one for leukocyte surface CD11b, and one labeled for leukocyte-platelet conjugates.

For determination of the surface density of GPIb, 100 μ l of sample was incubated with saturating concentrations of FITC-anti-GPIb (SZ2, AMAC) for 20 minutes, washed, and resuspended in 300 μ l Tyrode's-HEPES buffer for fluorescence-activated cell sorter (FACS) analysis.⁴ To measure the percentage of circulating platelets expressing P-selectin, a separate 100 μ l of sample was incubated with fluorescein isothiocyanate (FITC)-anti-GPIIb/IIIa (P2, AMAC) and biotinylated-anti-P-selectin (1E3) MoAb for 20 minutes, washed, and resuspended in 100 μ l Tyrode's-HEPES buffer. The sample was then incubated with saturating concentrations of PE-avidin (Becton-Dickinson) for 20 minutes, washed, and resuspended in 300 μ l Tyrode's-HEPES buffer for FACS analysis.²⁵

Leukocyte surface CD11b was measured by incubating 100 μ l samples with saturating concentrations of PE-anti-CD11b (D12, Becton-Dickinson) for 20 minutes. The samples were then washed and resuspended in 300 μ l Tyrode's-HEPES buffer for FACS analysis. For the percentage of leukocyte-platelet conjugates, a separate 100 μ l sample was incubated with saturating concentrations of FITC-conjugated anti-CD45 and biotinylated anti-GPIIb/IIIa (P2, AMAC) MoAb for 20 minutes, then washed and resuspended in 100 μ l Tyrode's-HEPES buffer. PE-avidin labeling and preparation for FACS analysis were performed as previously stated.

Flow cytometry. Samples were analyzed on a FACScan flow cytometer (Becton-Dickinson) with data stored in list mode files. The determination of the surface density of GPIb and the percentage of platelets expressing P-selectin was done as previously described.^{3,26,27} For GPIb, a single platelet gate using forward scatter was used to ensure that measurements of GPIb were not biased by microaggregate formation. The measurement of leukocyte surface CD11b was done by live-gating on leukocyte-sized events, using forward- versus side-scatter parameters, with distinction of PMN versus monocytes by the same criteria. Analysis for conjugates has been previously described.¹² As noted, isotype- and fluorochrome-matched control MoAb were used in each experiment to determine "non-specific" background MoAb binding. Statistical analysis included repeated-measures analysis of variance and, where appropriate, an unpaired *t* test.

Results

The demographics of the cyanotic and noncyanotic patients are shown in Table I. The two groups differed

Fig. 1. Changes in surface expression of platelet GPIb. Mean fluorescence of anti-GPIb antibody binding to platelets was measured in samples of whole blood taken before start of operation (*BASE*), 5 minutes after heparinization (*HEPARIN*), 10 minutes after start of operation (*10 MIN CPB*), shortly before separation from CPB (*END CPB*), and 1 to 2 hours after termination of CPB (*1H POST CPB*). Values are shown as mean $\pm$ 1 standard deviation. Noncyanotic patients are represented by black circles, cyanotic patients by black diamonds, and mean for all patients by open squares. GPIb decreased significantly at end of CPB and 1 to 2 hours after CPB compared with baseline values. In addition, surface expression of GPIb in cyanotic patients was significantly lower than that in noncyanotic patients ($p = 0.01$) for all time points.

Table I. Demographics of the cyanotic and noncyanotic patients

	Cyanotic (n = 7)	Noncyanotic (n = 6)
Age (mo)	15 (0.2-36)	14 (0.4-60)
Preop. hematocrit*	46 (38-53)	38 (33-41)
CPB time (min)*	177 (123-230)	79 (33-95)
Weight (kg)	8.1 (4-13)	9.9 (6.5-59)
12-Hour blood loss/weight†	26 (7-43)	15 (8-89)
RBCs transfused (ml)‡	56 (0-170)	32 (0-70)
Platelets transfused (ml)‡	50 (0-100)	32 (0-80)

Values are given as the mean and (in parenthesis) range. RBC, Red blood cells.

*Cyanotic group different from noncyanotic ($p < 0.05$).

†Chest tube drainage during 12 hours after operation/weight.

‡Milliliters transfused in first 24 hours after operation.

significantly in baseline hematocrit values and in the duration of CPB, with the cyanotic patients having significantly longer CPB times. Other variables displayed did not differ significantly. Chest tube drainage adjusted for weight showed a trend toward being greater in the cyanotic patients, but the difference was not significant ($p = 0.11$). Cyanotic patients also received more platelet transfusions in the perioperative period (six of seven cyanotic patients versus one of six noncyanotic patients), but this did not reach statistical significance ($p = 0.07$). In the cyanotic group, operations included two atrioventricular canal repairs, both with pulmonary artery

debanding and reconstruction; two fenestrated Fontan operations; an arterial switch with atrial septal defect closure; a tetralogy of Fallot repair; and a ventricular septal defect closure. In the noncyanotic group, three patients had patch closure of perimembranous ventricular septal defects, one including foramen ovale closure, and the other two also had primary closure of an atrial septal defect. One patient underwent a repeat aortic valvuloplasty, one a repeat transannular pulmonary artery patch (status after tetralogy of Fallot repair), and another a primary tetralogy of Fallot repair.

Platelet adhesion receptors. The average surface expression of the major platelet von Willebrand factor receptor, GPIb (Fig. 1), decreased during CPB, reaching significance at the end of CPB and decreasing further 1 to 2 hours after termination of CPB. The mean decrease in surface GPIb on single platelets fell to 75% of baseline values after CPB, and in selected patients GPIb decreased to as low as 63% of baseline values. Comparison of cyanotic with noncyanotic patients revealed comparable percentage decreases in GPIb during the perioperative period, but as demonstrated in Fig. 1, the baseline density of GPIb in the cyanotic patients was lower than that in their noncyanotic counterparts and remained lower for all time points during and after CPB. With the use of analysis of variance for repeated measurements, the difference between these patient groups was significant at $p = 0.01$.

Platelet activation. The percentage of circulating activated platelets, that is, the percentage expressing sur-

Fig. 2. Platelet activation with CPB. Percentage of circulating platelets expressing P-selectin was measured at time points described in Fig. 1. All values represent means $\pm$ Standard error of the mean for the 13 patients.

Fig. 3. Leukocyte-platelet conjugates with CPB. Percentage of leukocyte-platelet conjugates was measured in whole blood taken at time points described in Fig. 1. All values represent means $\pm$ Standard error of the mean for the 13 patients.

face P-selectin, increased over baseline values within 10 minutes of the start of CPB (Fig. 2). This percentage continued to rise during CPB, peaking at $25\% \pm 6.7\%$ (mean $\pm$ standard error of the mean for the 13 patients) of circulating platelets just before discontinuation of CPB. The percentage of P-selectin-positive platelets at 1 to 2 hours after bypass then declined, but remained significantly higher than baseline levels. Comparison of the cyanotic and noncyanotic patients revealed no difference in either the time course or the degree of platelet activation occurring during CPB. The absolute platelet count decreased by 67% between the preoperative sample and that obtained 1 hour after CPB. The platelet counts in

cyanotic and noncyanotic patients did not significantly differ at either time point.

Platelet-leukocyte binding. For the 13 patients, the percentage of leukocytes with bound platelets was comparable between leukocyte subsets before the start of operation, with $36\% \pm 2.8\%$ of monocytes and $35\% \pm 2.8\%$ of PMN binding platelets. The percentage of monocyte-platelet conjugates (Fig. 3) increased with the start of operation, then increased further at approximately 10 minutes after start of CPB with a mean of $66\% \pm 5.3\%$ of circulating monocytes having bound platelets ($p < 0.05$). This number remained essentially unchanged at the end of CPB and decreased slightly but

Fig. 4. Leukocyte CD11b with CPB. Fluorescence-labeling of CD11b was measured on monocytes and PMN in whole blood taken at time points described in Fig. 1 and are expressed as percentage of baseline fluorescence value. Each measurement is shown as mean $\pm$ standard error of the mean for the 13 patients.

remained significantly elevated over baseline binding at 1 to 2 hours after termination of CPB, where $56\% \pm 6.4\%$ of circulating monocytes had bound platelets.

The percentage of circulating PMN-platelet conjugates increased in the period before bypass, peaking at $46\% \pm 4.4\%$, markedly earlier and lower than peak monocyte-platelet binding. With initiation of CPB, the percentage of PMN-platelet conjugates declined, reaching significance at 1 to 2 hours after termination of CPB.

In a pattern similar to that of PMN-platelet binding, the percentage of circulating lymphocyte-platelet conjugates rose slightly in the period before bypass, then decreased significantly during CPB ($p < 0.05$), and remained significantly below baseline values at 1 to 2 hours after termination of CPB. There was no difference between cyanotic and noncyanotic patient groups in the numbers of circulating platelet-leukocyte conjugates at any time point.

Monocyte and PMN activation. Surface expression of CD11b on monocytes increased significantly ($p < 0.05$) during and after CPB, peaking 1 to 2 hours after termination of bypass (Fig. 4) at four times the baseline value. Expression of CD11b on PMN also increased significantly ($p < 0.01$) to a value five times that at baseline, but, unlike monocytes, PMN CD11b peaked earlier than monocytes at termination of CPB, then decreased in the early period after CPB. Cyanotic and noncyanotic patients demonstrated comparable increases in monocyte and PMN CD11b.

The quantitative relationship between changes in platelet-leukocyte binding and simultaneous changes in leukocyte-CD11b expression over time for individual patients was examined to determine whether there was a

temporal and quantitative correlation of monocyte and/or PMN CD11b expression and increased circulating platelet-leukocyte conjugates. With the use of linear regression analysis, at the point of peak monocyte CD11b expression (1 hour after CPB), the percentage of monocyte-platelet conjugates correlated with the monocyte CD11b expression ($r = 0.58, p = 0.04$). In contrast, there was an inverse relationship ($r = -0.49$) between the degree of PMN-platelet adhesion and PMN CD11b expression, which was not statistically significant ($p = 0.09$). The absolute leukocyte count decreased by 29% over the course of CPB. The absolute number of PMNs rose by 25%, whereas the number of monocytes and lymphocytes decreased by 79% and 67%, respectively. Cyanotic and noncyanotic patients did not differ significantly with respect to these changes.

Discussion

Children with CHD undergoing operation necessitating CPB may represent a population at particular risk for hemorrhagic complications. The large volume of the bypass circuit relative to the patient's intravascular volume implies that circulating cells spend a majority of their time in contact with this foreign surface. In addition, children with CHD, and in particular cyanotic CHD, are likely to have preexisting platelet defects that result in a prolonged bleeding time¹⁵⁻¹⁹ and whose impact on perioperative bleeding has been difficult to ascertain. This is the first clinical study to examine alterations in platelet adhesion receptors in addition to activation of platelets and leukocytes in children undergoing CPB for cyanotic and noncyanotic CHD. In addition, this study has examined the changes in platelet-leukocyte conjugates during

CPB and their relationship to platelet and leukocyte activation.

The platelet adhesion receptor, GPIb, is responsible for binding von Willebrand factor and is necessary for the initial adhesion of the platelet to damaged subendothelium under conditions of high shear stress. We and others have shown in adults that CPB results in a decrease in the surface expression of this adhesion receptor during CPB and continuing into the period after CPB.^{4,5} In this study we have shown that platelets in pediatric patients with CHD also demonstrate a decrease in GPIb during CPB, and the percentage decrease is comparable with that of the adult population.⁸ Comparison of cyanotic patients with their noncyanotic counterparts, however, revealed that at baseline and at every subsequent point, the surface expression of GPIb was lower in the cyanotic patients. This finding may explain the relatively high prevalence of a prolonged bleeding time in this population. Gil and associates²⁸ have shown that patients with noncyanotic CHD have a high prevalence of a deficiency of the largest von Willebrand factor multimers, which are most hemostatically effective. This has not been studied in cyanotic patients; however, if true in cyanotic patients as well, this together with the baseline GPIb receptor deficit and the added decrease incurred during CPB may put these patients at particular risk for hemorrhagic complications. The cyanotic patients in this study exhibited a trend toward more blood loss and a higher requirement for platelet transfusions. A larger study is needed to determine if this difference is real and whether it can be attributed to the lower levels of GPIb in this population, the longer CPB times, or some combination of these and other variables.

In addition to serving as a marker for platelet α -granule release, P-selectin has been demonstrated to mediate *in vitro* binding of activated platelets to monocytes and PMN.^{9,29} We and other investigators have previously demonstrated in adults that extracorporeal circulation activates platelets and causes an increase in the percentage of circulating platelets expressing P-selectin. This increase correlates with an increase in the percentage of monocyte-platelet conjugates and, to a lesser extent, PMN-platelet conjugates,⁸ reflecting the higher affinity of monocytes for activated platelets seen *in vitro*.¹²

In this study, unmanipulated blood drawn from an arterial catheter was immediately fixed in paraformaldehyde. Prior studies by our laboratory had shown that this procedure produces minimal (<5%) activation of platelets and appears to provide a reasonable approximation of "in vivo" platelet-leukocyte conjugates.¹² Although we cannot eliminate the possibility that the conjugate formation seen is an artifact of blood drawing or the stabil-

ity of the assay over time, the dependence of activated platelet-leukocyte adhesion on both divalent cation and the G1 epitope of the P-selectin molecule in normal whole blood^{9,12,28} and the reproducible change observed over time in this study strongly suggest that these results reflect the *in vivo* situation.

There was a steady increase in both platelet activation and monocyte-platelet conjugate formation, the latter peaking 10 minutes after the start of CPB and remaining elevated during and 1 to 2 hours after CPB. PMN-platelet binding also rose in the period before bypass, then abruptly decreased with onset of CPB. The leukocyte receptor for P-selectin is not definitely known,^{10,30-33} but on PMN appears to be a labile one that decreases with PMN activation.³⁴ The decrease in PMN-platelet adhesion with onset of CPB is consistent with the competitive advantage monocytes have over PMN, but may also reflect loss of the P-selectin receptor. The inverse relationship between PMN-platelet adhesion and PMN activation as measured by CD11b supports this latter explanation. Lymphocyte-platelet conjugates also decreased on bypass. This finding parallels *in vitro* observations that most lymphocytes do not bind P-selectin-positive platelets¹¹ and, furthermore, that the platelet activation and subsequent adhesion to monocytes, along with the formation of platelet-platelet aggregation, can compete platelets off lymphocytes.¹² This pattern of platelet activation and parallel formation of monocyte-platelet conjugates is comparable with that found in adults undergoing CPB. The percentage of activated platelets was slightly higher in this group of pediatric patients than in the adults (25% platelet activation versus 19% in adults), and the percentage of monocytes with bound platelets was significantly higher (66% of monocytes with bound platelets in the pediatric group versus 44% in adults, $p < 0.05$).⁸ These differences may reflect the increased blood volume exposed to the extracorporeal circuit relative to the intravascular volume in children or, possibly, the increased time on bypass. Alternatively, they may represent an intrinsic difference in pediatric versus adult platelets.

CD11b on monocytes and PMN increased over time on bypass, but with slightly different time courses. PMN expression of CD11b peaked at termination of CPB at five times its baseline values, whereas monocyte expression rose more gradually and peaked 1 to 2 hours after CPB at four times baseline values. As was seen in the adult population, monocyte CD11b expression correlated with monocyte-platelet conjugate formation, whereas PMN CD11b did not. The functions of CD11b are particularly relevant to CPB. CD11b is capable of binding C3b, fibrinogen, fibronectin, and factor X.³⁵ In animal

models of reperfusion injury, infusion of anti-CD11b MoAB decreases PMN accumulation at the site of injured myocardium and increases tissue recovery.³⁶ Furthermore, the addition of MoAb to monocytes in vitro results in marked interference with monocyte adherence to endothelial monolayers.³⁷ Another factor that may contribute to tissue injury on CPB is classic and contact activation of the complement system.³⁸ CD11b may play a critical role in regulation of complement effects inasmuch as it functions as the receptor for the inactivated complement component C3b.³⁹

The complications that may occur after CPB include bleeding,⁴⁰ pulmonary dysfunction, and other organ dysfunction.⁴¹ These may be due to a combination of cellular and soluble factor changes induced by CPB. One possible consequence of increased leukocyte-platelet adhesion is that such conjugates may be physically sequestered in the pulmonary or other localized vascular bed, producing local vasoactive changes, inflammation, or organ dysfunction.⁴² It is also possible that platelet adhesion to leukocytes induces leukocyte activation and subsequent elaboration of cytokines,⁴³ which may cause pulmonary and systemic endothelial changes, leading to hypoxemia and tissue injury. Finally, platelet-leukocyte adhesion may result in enhanced monocyte procoagulant activity via increased tissue factor expression.¹⁴ We believe it is unlikely that leukocyte-platelet conjugate formation contributes to the platelet defect associated with CPB.⁴⁴ Because the activated platelets bind through P-selectin and therefore have undergone α -granule release, their contribution to secondary aggregation is limited even if they remained free in the circulation.

CD11b upregulation on monocytes could be a result of (1) direct mechanical activation of leukocytes by the CPB apparatus or exposure to nonphysiologic biomaterials, (2) complement activation,⁴⁵ or (3) as an indirect consequence of platelet activation followed by leukocyte-platelet adhesion and subsequent leukocyte activation. Our in vitro data suggest that platelet adhesion to monocytes is one signal that can result in upregulation of monocyte CD11b expression in whole blood and in fractionated cell assays. The time course on CPB of CD11b expression on monocytes is similar to that in the in vitro data, and the correlation between platelet-monocyte binding and monocyte CD11b expression on CPB in adults and now in the pediatric population supports the possibility of upregulation of CD11b as a result of platelet binding. The absolute platelet and monocyte counts were decreased in the early period after bypass, coincident with peak monocyte CD11b expression and elevated platelet activation and platelet-monocyte adhesion. It is possible that plate-

let-monocyte conjugate formation and increased expression of CD11b on monocytes contributes to the egress of platelets and monocytes from the circulation, but this cannot be confirmed from the present study. By contrast, our in vitro data showed that PMN-platelet conjugate formation was not accompanied by increased PMN CD11b expression. However, on CPB, the insult from extracorporeal circulation and complement activation may act directly to induce CD11b expression on PMN. We hypothesize that all three mechanisms are operative in CPB, with direct effects of the apparatus and complement activation possibly more critical to the PMN.

In summary, the changes in platelet and leukocyte adhesion receptors and platelet-leukocyte conjugate formation induced by CPB are qualitatively similar in pediatric patients with CHD to those in adults undergoing CPB for coronary artery bypass grafting. Of note, however, the cyanotic subgroup may be at particular risk for bleeding as a result of the decrease in the von Willebrand factor receptor on platelets induced by CPB superimposed on a baseline deficit in this receptor. These studies help form a basis for future examination of the benefits of various prophylactic and therapeutic interventions to prevent hemorrhagic and inflammatory complications in this patient group.

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