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Does aprotinin preserve platelets in children with acyanogenic congenital heart disease undergone surgery with cardiopulmonary bypass?

Aprotinina preserva plaquetas em crianças com cardiopatia congênita acianogênica operadas com circulação extracorpórea?

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Abstract

Objective: **Evaluation of the hemostatic and platelets effects in children with acyanogenic congenital heart disease undergone on-pump surgery who received aprotinin.**

Methods: **A prospective randomized study was performed on children aged 30 days to 4 years who had undergone correction of acyanogenic congenital heart disease using cardiopulmonary bypass (CPB) and were divided into two groups: Control (n=9) and Aprotinin (n=10). In the Aprotinin Group the drug was administered before and during CPB and the hemostatic dysfunction was analyzed by clinical and biochemical markers. Differences were considered to be significant when *P*<0.05.**

Results: **The groups were similar regarding demographic and intraoperative variables, except for a greater hemodilution in the Aprotinin Group. The drug presented no benefit regarding time of mechanical pulmonary ventilation, stay in the postoperative intensive care unit and hospital, or regarding the use of inotropic drugs and renal function. Platelet concentration was preserved with the use of Aprotinin, whereas thrombocytopenia occurred in the Control Group since the initiation of CPB. Blood loss**

Note of the Editor:
The article "Does Aprotinin preserve platelets in children with acyanogenic congenital heart disease undergone surgery with cardiopulmonary bypass?" is being published, despite its removal from the market because it presents important data in respect to such drug and its action.

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was similar for both groups. There were no complications with the use of Aprotinin.

Conclusion: **Aprotinin quantitatively preserved the blood platelets in children with acyanogenic congenital heart disease.**

Descriptors: **Extracorporeal Membrane Oxygenation. Aprotinin. Hemostasis. Blood Platelets. Cardiovascular Surgical Procedures.**

Resumo

Objetivo: **Avaliação dos efeitos hemostáticos e plaquetários em crianças submetidas a correção de cardiopatias congênitas acianogênicas com circulação extracorpórea que receberam aprotinina.**

Métodos: **Estudo prospectivo randomizado em crianças de 30 dias a 4 anos de idade, submetidas a correção de cardiopatia congênita acianogênica, com circulação extracorpórea (CEC) e divididas em dois grupos, um denominado Controle (n=9) e o outro, Aprotinina (n=10). Neste, a droga foi administrada antes e durante a CEC. A disfunção hemostática foi analisada por marcadores clínicos e bioquímicos. Foram consideradas significantes as diferenças com *P*<0,05.**

Resultados: **Os grupos foram semelhantes quanto às variáveis demográficas e intra-operatórias, exceto por maior hemodiluição no Grupo Aprotinina. Não houve benefício quanto aos tempos de ventilação pulmonar mecânica, permanência no centro de terapia intensiva pediátrica e hospitalar, nem quanto ao uso de inotrópicos e função renal. Ocorreu preservação da concentração plaquetária com a Aprotinina, enquanto no grupo Controle houve plaquetopenia desde o início da CEC. As perdas sanguíneas foram semelhantes nos dois grupos. Não houve complicações com o uso da Aprotinina. Conclusão: A Aprotinina preservou quantitativamente as plaquetas em crianças com cardiopatia congênita acianogênica.**

Descritores: **Circulação extracorpórea com oxigenador de membrana. Aprotinina. Hemostasia. Plaquetas. Procedimentos cirúrgicos cardiovasculares.**

INTRODUCTION

The morbidity and mortality of cardiopulmonary bypass (CPB) is, in most of cases, due to the limited biocompatibility of materials, triggering multiple organ system dysfunction after infusion, characterizing the inflammatory response syndrome (SIRS), including bleeding and coagulopathy, by the activation of coagulation and fibrinolysis systems [1,2].

Although mostly of adults recovers well, children with low body weight, particularly newborns and infants often have complications. These complications determine exposure to a greater number of donors of blood, prolonged time of hemostasis in the operating room, need for delayed sternal closure, with consequent prolongation of mechanical ventilation and stay in the Pediatric Intensive Care Unit (PICU) and worrying morbidity and mortality associated with higher hospital costs. Strategies to anticipate them, avoid them or fight them are of intense interest, especially the preoperative scores of risk stratification, the anesthetic and surgical refinement, and the optimization of CPB circuits. The use of Aprotinin (Trasylol®, Bayer Pharmaceuticals Corporation), a non-specific serine proteases inhibitor, consisting of the polypeptide chain of 6512 Daltons, hydrophilic and basic, was one of these pharmacological strategies, whose hemostatic property reduces blood loss after cardiopulmonary bypass procedure [1-3]. Recently, in 2006, Arnold et al. [4], in a meta-analysis of 12 randomized studies involving 612 children undergoing CPB, observed, with Aprotinin, 33% reduction in the rate of transfusion.

Pediatric studies with Aprotinin were unduly difficult to interpret [5], due to the wide variation in dosage, a high variability in metabolism and antifibrinolytic action of Aprotinin in neonates, the hemodilution, the dispersion at the age of the cohort studied and the various surgeries performed , with consequent variability in time and in the management of CPB and surgery [2,5]. The first studies date from the 80's, with Masiaka et al. [6] and those published in the 90's conflicting with several pediatric placebo-controlled studies, reporting low intra- and postoperative bleeding. The Aprotinin reduced the number of blood transfusions, use of blood products, length of operating room, time to sternal closure, length of hospital stay and hospital expenses in reoperations with CPB, especially in cases of Jatene's surgery and in cyanotic patients [4.7]. Mössinger et al. [2] in 2003 has shown that the drug reduces levels of hemostatic and pulmonary dysfunction.

It was not shown increased myocardial infarction (MI), renal failure (ARF) and stroke (CVA) with Aprotinin in adults until a non-randomized observational study reported the opposite, establishing a higher mortality at 5 years after surgery. The manufacture of Aprotinin was suspended in November 2007. The results of the Canadian BART study (Blood Conservation using Anti-fibrinolytics: a Randomized Trial in a cardiac surgery population study) confirmed the increased risk of mortality with Aprotinin use in adults [8].

Many centers routinely employed Aprotinin in pediatric cardiac surgery with CPB. In recent retrospective cohort studies of children undergone on-pump surgery, there was no association between use of Aprotinin and ARF, dialysis,

neurologic complications and mortality [9]. The application of safety data in adults to pediatric practice requires caution and it is of questionable validity because such populations are distinct, and drugs that have a higher risk of complications in adults can be safe in children, and vice-versa. The perspective for future randomized and controlled pediatric studies with Aprotinin has shown to be limited currently [10].

Based on the aforementioned situation, this study was performed in an attempt to demonstrate the hemostatic usefulness of Aprotinin on a subgroup of acyanotic children with high prevalence in Pediatric Cardiology Services, but which has received little attention from researchers.

The aim of this study is to verify the hemostatic and platelet effects of the intraoperative administration of Aprotinin in children undergoing correction of acyanogenic congenital heart diseases with CPB.

METHODS

Patients

We studied 19 children of both genders, who had undergone correction of acyanogenic congenital heart disease using CPB from January to December 2004. The patients were divided randomly into two groups: Aprotinin (n=10) and Control group (n = 9). The study was prospective and not a blind one. Inclusion criteria were elective surgeries and age between one month and four years. The exclusion criteria were: cardiovascular surgery prior exposure to Aprotinin within six months prior to surgery, use of salicylates up to 7 days before surgery, allergic immune disorders, hepatic, renal or coagulation disorders and episodes of cardiac arrest, sepsis, vasculitis, less than two months ago. Parents or legal guardians signed written informed consent. The study was approved by the Human Research Ethics Committee of the HC-FMRP-USP under Process No. 6665/2004.

Methodology

The distribution of patients in both groups, was performed randomly, after induction of anesthesia.

Anesthetic and surgical techniques

In pre-anesthesia room, midazolam was administered peripherally. In the operating room, the children were placed in the supine position on warming blanket and under a flow of heated air, monitored with electrocardiogram (ECG) and pulse oximetry. Anesthesia consisted of intravenous injection (IV) using midazolam, fentanyl or sufentanyl, and muscle relaxation with vecuronium or pancuronium. Anesthesia was maintained with continuous IV infusion of fentanyl or sufentanyl associated with inhalation of isoflurane. After tracheal intubation, it was initiated mechanical ventilation. Invasive monitoring of blood pressure (BP) was performed by percutaneous radial or femoral artery. The right internal jugular or femoral vein were cannulated for fluid infusion and control of central venous pressure (CVP) and oropharyngeal and peripheral temperatures (foot sole) were recorded.

Diuresis was measured by delayed vesical catheterization. Caudal epidural anesthesia with morphine and clonidine was performed according criterion of the anesthesia team (three patients in each group). Steroids were not used. After anesthesia, it was administered Amikacin 7.5 mg/kg IV, associated with Cefazolin (40 mg/kg IV) in children hospitalized for less than 48 hours. In the other, the association was with Vancomycin (10 mg/kg IV). Additional dose of Vancomycin (5 mg/kg) was added to the reservoir of the oxygenator. The Aprotinin (Trasylol®, Bayer, Leverkusen, Germany) at a dose of 240mg/m² IV, was infused for 20 to 30 minutes, from the beginning of the surgical incision, followed by continuous infusion of Aprotinin of 56mg/m²/h until complete the healing.

The drug (240 mg/m²) was also added to the perfusate of the oxygenator. Ten minutes before the infusion (IV) of Aprotinin, sensitivity test was performed with minidose of 10,000 UIC (KIU = kallikrein inhibitory units. Under topical antisepsis with povidone iodine (PVP-I), a median sternotomy and total thymectomy were performed. Children were heparinized (3 mg/kg IV) (Heparin sodium, Roche, Basle, Switzerland), under control of Activated Clotting Time (ACT) (Hemotec ACT II®, Medtronic, Englewood, CO, USA), in order to maintain ACT above 480 seconds in both groups (with additional doses of heparin, 0.5 mg/kg IV).

The ascending aorta and the vena cavae were cannulated through purse-string sutures with polypropylene yarn. In CPB, performed with passive venous drainage, we used a hollow fiber membrane oxygenator. Roller pumps with arterial flow in normothermia, 2.5 l/m²/min were used. Oropharyngeal temperature, in the CPB was reduced to 28°C for heat exchange in the oxygenator. The perfusate was calculated to result in hematocrit (Hto) of 30% and consisted of red blood cells, Ringer's solution, fresh frozen plasma, Manitol 20% (4 to 5 ml/kg), heparin sodium 1mg/10 ml of blood product and sodium bicarbonate at 8.4% 1mEq/kg.

In the Aprotinin group, the volume of drugs added to the perfusate was included in the calculation. Hemoconcentrators with polyethersulphone membranes were used for ultrafiltration, started during reheating. The Hto during CPB was maintained by addition of red blood cells. After aortic clamping, anterograde hyperkalemic cardioplegia (10 ml/kg) at 4°C, suspended one meter high from the operating table, was infused passively, in the aortic root and repeated every 30 min. The first dose was

crystalloid and the remaining blood ones. The systemic reheating up to 37°C, was started simultaneously with the infusion (0.5 to 1.5 μg/Kg/min) of sodium nitroprusside. When finishing the CPB, the cannulas from aorta and superior vena cava were removed, and the arterial line was connected to the cannula of inferior vena cava.

The volume in the oxygenator was ultrafiltrated, in the intervals between the replacement of volume, and infused by cannula from the inferior vena cava. It was administered protamine hydrochloride (ICN Pharmaceuticals Ltd., Valeant Pharmaceuticals International, USA), 1:1 ratio, in relation to the total dose of heparin used, and the return of ACT to baseline levels was confirmed. The remaining blood in the CPB circuit was recovered on transfer bags without anticoagulant for IV infusion by venous catheter after decannulation. The pericardium was closed, if it may not generate hemodynamic instability. In children up to 10 kg, the sternal synthesis was performed with polyglactin sutures and the others steel wire 1 was used. Mediastinal vented drain was implanted under aspiration of 20 cm of water.

Preoperative clinical characteristics

We assessed demographic variables (age, gender, weight, height and body surface area) and calculated the risk categories according to RACSH-1 and Aristotle Basic scores as well as Ross and Reithmann scores modified to congestive heart failure (CHF). Ongoing medications, arrhythmia, electrocardiogram, chest radiograph, Doppler echocardiogram, cardiac catheterization and surgical diagnosis were recorded and hemodynamic variables were documented (pulmonary and systemic arterial pressure), blood count, coagulation tests and other tests such as of renal function (urea and plasma creatinine) and hepatic test (glutamic-oxaloacetic transaminase - SGOT and bilirubin) were obtained.

Surgical data

Data were collected such as: surgery performed, intracardiac access route, duration of surgery, anesthesia and CPB, aortic clamping time, oropharyngeal minimum temperature, fluid balance, diuresis, volume of packed red cells and fresh plasma and packed platelets; TCA before, during and after CPB and complications.

Postoperative clinical conditions

Patients were treated according to protocol of postoperative treatment. Retrospectively, we calculated the index recently proposed by Mattos et al. [3] in 2006. The times of use of inotropes, IV vasoactive drugs, nitric oxide, and stay in the PICU and elapsed time until discharge or death were measured as well as the duration of mechanical ventilation (MV). Postoperative bleeding accumulated in 4, 12, 24 and 48h, and the use of blood products, at 6 and 24 hours postoperatively, were expressed in ml/kg, and the number of donors that patients were exposed was recorded.

Biochemical and hematological assessment

Arterial blood samples (3 ml) were collected at the following times: T1: After induction of anesthesia, before the administration of Aprotinin; T2 - 15 min after the start of CPB, T3 – Immediately before the end of CPB; T4 - Five minutes after protamine administration; T5 - Four hours after T4; T6 - Twelve hours after T4; T7 - Twenty-four hours after T4; T8 - Forty-eight hours after T4. The prothrombin time (PT), according to the INR (International Normalized Ratio) and activated partial thromboplastin time (aPTT) were measured, as well as routine exams measuring the ion concentrations of sodium, potassium and calcium, urea, creatinine, MB fraction of creatine phosphokinase (CPK), SGOT, bilirubin, gasometry and serum lactate levels. The rates of hemoglobin (Hb) and hematocrit (Hto) and platelet count were observed in the time T1 to T8. Albumin was measured in plasma by colorimetric method, by spectrophotometer BAUSCH & LOMB (Model SPECTRONIC 70.1, USA), at times T1 to Tn (T2 to T4) (normal range of 3.5 to 5.5 g/dl).

Statistical analysis

Qualitative variables were expressed in relative and absolute frequencies. Quantitative values were expressed by the minimum and maximum values, mean, median and standard deviation. The comparison between groups by analysis using mixed model (PROC MIXED® pack, SAS/STAT® software, version 9, SAS Institute Inc., Cary, NC, USA) assumed normal distribution, and was complemented by the coefficient of correlation (PROC CORR® pack, SAS/STAT® software, version 9, SAS Institute Inc., Cary, NC, USA). In the comparisons of subgroups, as well as in intra- and intergroups, we used the nonparametric exact Wilcoxon test (Software R Development Core Team (2005). R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org). Figures depicting boxes, the horizontal limits, represent the 25th and 75th percentiles, the line inside the box represents the median and the limits and lines outside the boxes reflect the variance of the variable. The full point expresses the mean, and the empty points, the outliers. The level of significance was 5%.

RESULTS

In comparisons between means and standard deviations of the groups, the means of the Aprotinin group preceded. In the figures, the statistically significant differences intragroups with respect to T1, are indicated by *, and intergroup by **.

Preoperative

The groups were similar in age and anthropometric variables (Table 1). Except as Ross score, modified, slightly more pronounced in the Aprotinin group (median 5.5 vs 3), RACHS-1 (median 2) and Aristotle Basic (median 6) were identical in both groups. The cardiac malformations were similar in both groups (Table 2), as well the surgeries performed.

Table 1. Age (d), weight (g), height (cm) and body surface (m2) (mean ± standard deviation), from Aprotinin and Control groups. The median is within brackets.

	Group	
	Aprotinin (n=10)	Control (n=9)
Age (d)	300 ± 406 (123)	286 ± 449 (132)
Weight (g)	6067 ± 3714 (4665)	6173 ± 4009 (4800)
Height (cm)	63 ± 16 (59)	63 ± 14 (60)
Body surface (m^2)	0.31± 0.14 (0.26)	0.32 ± 0.16 (0.27)

Intraoperative

In the Aprotinin and Control groups, the duration of the surgery (192.5 ± 39.3, median = 185 min vs. 194.44 ± 35.30, median = 210 min), anesthesia (319.5 ± 39.04, median = 325 min vs. 288.33 ± 32.01, median = 270 min), CPB (64 ± 21.05, median = 62.50 vs. 69.44 ± 10.13, median = 70 min), aortic clamping (39 ± 17.12, median = 36 min vs. 44.11 ± 7.11, median = 45 min), and the minimum oropharyngeal temperature during CPB (28.9 ± 2.1, median = 28.7° C vs. 28,2 ± 2.7, median = 29.1° C) were similar. Oxygenators had similar distribution in groups. The total volume of perfusate and supplements were slightly higher in the control group (384 ± 185 ml vs. 430 ± 206 ml). The estimated blood volume was similar in both groups. The total amount of aprotinin administered was 177.56 ± 77.76 mg, corresponding to 126.84 ± 55.55 ml of Trasylol.

The total amount of heparin used was similar (39.2 ± 15.3 mg, median = 33.5 mg vs. 39.0 ± 16.7 mg, median = 30 mg), and corresponded, on average, to 7.4 ± 2.9 vs. 7.1 ± 3.8 mg/kg of body weight. The use of red blood cells in CPB (221 ± 55 ml vs 248 ± 73 ml) and hemofiltered volume (378 ±

Table 2. Age (days), gender, weight (g), Ross, RACHS-1 and Aristotle Basic preoperative scores and clinical-surgical index by Mattos of the patients undergone surgery with the respective diagnoses.

Patient No. order	Age (days)	Gender	Weight (g)	Ross	RACHS-1	Arist	Mattos	Pre-and intraoperative diagnosis
				APROTININ GROUP				
1	137	F	4300	7	2	6	6	IVC + PDA
2	47	M	3450	5	2	6	6	IVC + IAC + PDA
3	189	M	5150	1	3	10,3	8	DOLV + PDA + PFO
4	519	M	8175	0	2	5,6	3	PVS + PDA
5	86	M	3320	7	3	10,3	8	DOLV + PDA
6	408	M	9000	8	2	6	4	IVC + *PA banding + PFO
7	1366	M	15000	1	2	4	1	AVSD partial IAC + IAC + PDA
8	110	M	5030	4	2	6	5	IVC +PDA + PFO
9	109	F	3650	7	2	9	7	multiple IVCs + IAC + PDA
10	33	M	3600	6	2	5,6	5	PVS + PDA + PFO
				CONTROL GROUP				
1	256	F	6870	2	2	6	5	IVC + PDA
2	37	M	2200	6	2	6	6	IVC + IAC + PDA
3	121	M	4800	3	2	6	6	IVC
4	242	F	6650	2	1	3	4	IAC
5	1468	F	15800	0	2	6,3	1	IVC + SubAo M + PDA
6	63	F	2890	5	2	6	6	IVC + IAC
7	185	F	7310	2	2	6	4	IVC
8	77	M	4520	8	2	6	5	IVC
9	132	M	4520	6	2	6	6	IVC + PFO

*Arist – Aristotle; F – female; M – male; IVC – interventricular communication; IAC – interatrial communication; PDA - persistent ductus arteriosus; DOLV - double outlet left ventricle; PVS – pulmonary valve stenosis; PA – pulmonary artery; PFO - patent foramen ovale; AVSD - atrioventricular septal defect; SubAo M – subaortic membrane. * PA banding = ligation of persistent ductus arteriosus surgically performed at 18 years of age*

244 ml vs. 335 ± 319 ml) was similar. Diuresis (ml/kg) of the two groups before (4.60 ± 4.39 vs. 2.64 ± 2.64), during (19.22 ± 18.53 vs. 18.13 ± 17.81) and after (21.22 ± 31.64 vs 11.93 ± 5.66) CPB was similar. The closing time of the chest was overlapping (28.5 ± 9.1 min vs. 28.8 ± 17.4 min). As the intraoperative blood balance, there was a strong tendency to negative balance in the Aprotinin group (-12 ± 166 ml, median = 15 ml vs. 125 ± 81 ml, median = 150 ml).

Postoperative

The water balance on admission of PICU were similar in both groups (24 ± 77 ml/kg, median = 25 ml/kg vs. 31 ± 32 ml/kg, median = 35 ml/kg). The two groups were similar in the clinical-surgical rate by Mattos (5.3 ± 2.2, median = 5.5 vs. 4.7 ± 1.6, median = 5). 70% of children in the Aprotinin group and 88.8% of the Control group were in the intermediate risk category (aged between 1 month and 1 year). Protein-energy malnutrition occurred in 90% vs 77.7% of children, and in almost all, with high-risk criterion (below the 5th percentile). By the presence of heart failure, pulmonary hypertension and/or genetic syndrome (clinical risk factors associated), nearly half (50% vs 55.5%) of children in both groups were high risk. All children in the Control group were in category of low risk for surgical complexity (Aristotle basic score), occurring in 80% of the Aprotinin group. In respect to the duration of CPB, all patients except for one of the Aprotinin group that exceeded 90 min, were of intermediate risk.

There were no significant differences between intergroups in times of inhaled nitric oxide (216 vs. 118 h), mechanical ventilation (79.50 ± 90.55, median 36 h vs. 120.73 ± 79.77 h, median 16h), stay in the PICU (6.4 ± 4.92, median = 5.5 vs 4.86 ± 4.80 days, median=3 days) and hospital stay (9.30 ± 4.99, median=7.5 vs 10.22 ± 11.68 days, median=5 days). The groups were not different as the time of use of inotropic agents (122.40 ± 118.36 m, median=132 h vs 78.55 ± 89.40 h, median=48 h).

Bleeding in the first 48 hours postoperatively was similar in both groups (17.6 vs. 18.1 ml/kg). One patient in the Aprotinin group was transfused with packed red blood cells (10 ml/kg) in T5, due to anemia, according to the PICU protocol. There was a tendency for greater infusion of human albumin in the Aprotinin group (27.58 ± 30.27 vs 12.95 ± 18.58 ml/kg). Platelet concentrate (12 ml/kg) was used in two patients in the Control group (T6 and T7, respectively). Children were exposed to donors of blood products and this number was similar in both groups (median 2).

Rates of baselines Hb (9.6 ± 1.1 vs. 8.9 ± 1.3) and Hto (29.3 ± 2.8 vs. 27.5 ± 4.2%) were slightly lower in T1, in the Control group. Both presented a significant decrease from T1 to T2 (7.6 ± 1.9 vs. 9.59 ± 1.5 g/dl and 23.4 ± 5.6 vs. 29.5 ± 3.9%), succeeded by progressive hemoconcentration up to T5 (12.1 ± 1.9 vs. 12.7 ± 2.9 g/dl and 37.6 ± 4.9 vs. 40.2 ± 8.6%), whose value was statistically higher than T1. In T2 and T3, Hb and Hto were significantly lower in the Aprotinin group (Figs. 1 and 2).

Platelet count (no°/mm³) in T1 was similar in both groups (183500 ± 86,053 vs. 250,777 ± 59736). With the start of CPB (T2), there was a decrease in both groups with nadir in T4 (106700 ± 56,631 vs. 82,211 ± 44655), or that is, five minutes after protamine administration. In the Control group, the drop remained statistically significant from T2 to T8 (except for T7). From T2 to T5, the platelet count was significantly higher in the Aprotinin group (Fig. 3). With 48 hours of postoperative (T8), the platelet count tended to recover only in the Aprotinin group (141333 ± 31878), while in the Control group such count remained significantly lower than in T1.

There was a statistically significant increase in aPTT in Aprotinin group in T5 (2.64 vs. 1.56). In both groups, it remained slightly increased in T6. A significant increase in INR in both groups at T5 (1.97 vs. 1.61), with significant difference between groups. In T6, the INR returned to normal value in the Control Group (INR = 1.32), but remained significantly high in the Aprotinin group (INR = 1.74). There were no deaths. All patients were discharged in good clinical condition and healing. Doppler echocardiographic assessment showed good surgical outcome in all patients. There were no allergic problems, hypotension, nor thrombosis, or adverse effects of Aprotinin.

Fig. 3 - Platelet count (n° x 10³/mm³) in the Aprotinin and Control groups. Statistically significant intra- and intergroups differences are distinguished.

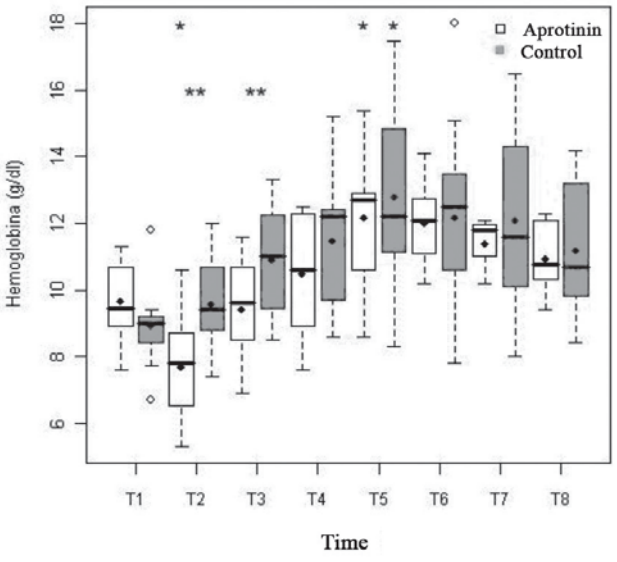

Fig. 1 – Hemoglobin rates (g/dl) in Aprotinin and Control groups, at times T1 to T8. The statistically significant differences are distinguished

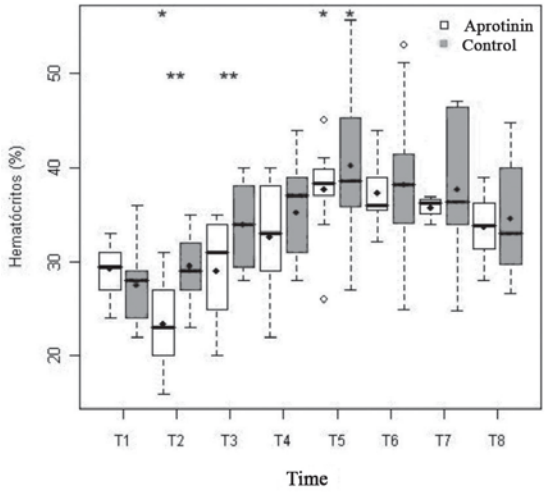

Fig. 2 – Hematocrit rates (%) in Aprotinin and Control groups, at times T1 to T8. The statistically significant differences are distinguished

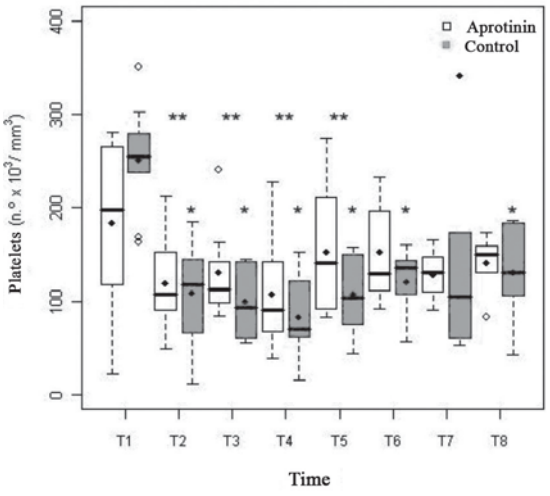

Fig. 3 – Platelet count (n° x 10³/mm³) in Aprotinin and Control groups. The statistically significant intra- and intergroups differences are distinguished

DISCUSSION

The use of Aprotinin in heart urgery was surrounded of discussion regarding the cost-benefit, since many believed that the cost and possible adverse effects contraindicate its use in cases of lower risk and it was not considered a drug of first choice, however, its use was a routine in several groups [9]. However, there was also a few doubts about its potential usefulness in children predisposed to excessive bleeding after surgery [4,5], such as those who had undergone surgery in Brazil, those of body weight lower and lower, usually due to an important degree of malnutrition. These considerations gave rise to this investigation in acyanotic children undergone on-pump surgery, on whom no benefit was observed regarding bleeding and need for transfusion, but an interesting quantitative preservation of platelets.

The age range was restricted to the period from 30 days to four years of life, in order to reduce the dispersion of age, which overshadows similar studies [2]. Newborns were excluded due to the greater propensity to complications of CPB secondary to severe neuro-endocrine response to tissue injury [3].

Despite the estimated mortality, both in-hospital or within 30 days, from 1 to 5%, based on risk scores more widespread (RACHS-1 and Aristotle), no deaths occurred in this study. The good surgical outcome was reinforced by the clinical-surgical index, more recently, proposed in Brazil by Mattos et al. [3] in 2006, whose scores (5.5 vs 5) correspond to the risk of death from 11.70% to 23.98%, due mainly to the prevalence of low body weight, indicative of malnutrition, and genetic syndromes, pulmonary hypertension and congestive heart failure (CHF), which certainly influenced morbidity.

Since it is believed that Aprotinin reduces bleeding and need for transfusion by dose-dependent mechanism [2-5], we chose by the use of the pioneer protocol (Hammersmith Hospital), entitled as a high-dose, proportional to body surface, in order to improve clinical benefit. Unfortunately, the dosage, certainly, had the side effect of introducing a greater hemodilution bias in the treated group, since the volume of perfusate, hemofiltrated and cardioplegia, as well as of red blood cells, diuresis and times of infusion and anoxia were similar to those from Control groups. The similarity of total postoperative bleeding between Aprotinin and Control groups (17.62 vs 18.11 ml/kg/ 48h) was unexpected and probably comes from the commitment to detailed hemostasis in the intraoperative perid and the lower risk of bleeding in patients with acyanotic heart disease [11]. The volume of postoperative bleeding is lower than reported in the literature [7,12]. Although a child of 15 kg from the Aprotinin group has been transfused (Hb = 8.6g/ dl), his bleeding was very small and the behavior followed the PICU protocol.

This reinforces the well-known critics to protocol of thresholds transfusion, among which those on hindering the comparison of published researches. There were no thrombotic presentation, or evidences of insufficient heparin in CPB. This finding is consistent with the institutions of great experience using Aprotinin in heart surgery [4,9,12].

The most common causes of bleeding after CPB include platelet dysfunction and hyperfibrinolysis [1], considering that the former is more important [12], and likely to exacerbation of thrombocytopenia, typical of CPB with hemodilution, by the adhesion and consumption in the cardiopulmonary bypass circuit and the platelet effects of heparin [13]. In this study, Aprotinin preserved quantitatively the circulating platelets, whereas the Control group developed with thrombocytopenia from the start of CPB until the end of the study in order to corroborate the findings of Masiak et al. [6] and Royston et al. [14]. As platelets suffer direct mechanical damage by shear forces and are sequestrated by various organs, by preserving platelet number through Aprotinin may also have resulted from attenuation of the sequestration and accumulation of platelets in the microcirculation [15]. The preservation of the circulating concentration of platelets due to the lower microcirculatory sequestration has a salutary effect, reducing the risk of vascular thrombosis, microembolization and vasoconstriction associated with CPB and has been determined both by inhibiting the activation of protease-activated receptor (PAR), and the production of thromboxane. As a result, platelet aggregation mediated by thrombin is blocked [16,17].

It is known that the adhesion of plasma proteins such as fibrinogen, to the surface of the CPB circuit, promotes strong activation and local platelet deposition. For this reason, Wendel et al. [18] in 1999, argued that high-dose Aprotinin may compete in the coating of the CPB circuit, making it more biocompatible and thus favoring the maintenance of platelet population and its viability in order to contribute to hemostasis after CPB. Moreover, the affinity of Aprotinin by proteins GPIIb/IIIa from the platelet receptor is cytoprotective, because it prevents the binding of platelets to foreign surfaces of the CPB circuit [19]. It is also known that Aprotinin reduces the binding of heparin on platelets. As a result, the platelet dysfunction induced by this anticoagulant is minimized by the drug [1] and can even be restored the ability of adhesion of the platelet that were rendered dysfunctional by heparin [20]. Due to reduction of platelet count in hypothermic CPB [19], one suspects, also, that the platelet-protective action of Aprotinin is more substantial [21] in this type of procedure, but it can also be functionally useful in preserving platelets even in normothermia [1].

It is important to emphasize that in the Control group, two patients (22%) who presented heavier bleeding, one by mediastinal drain (49.97 ml/kg) and the other by the vascular accesses, presented thrombocytopenia (70,000 and 73,000 platelets/mm^3, respectively) and needed to receive platelet transfusion. The use of platelet concentrate was reported to be reduced in newborns and even to those undergone Jatene's surgery, as noted by Williams et al. [22] and Murugesan et al. [7] in 2008, as well as the rate of surgical re-exploration for bleeding in the postoperative period in children below 20 kg, as reported by Breuer et al. [23] in 2009. The dramatic reduction of platelets subsequent to the infusion of protamine, although greater in the Control group, reflects that the platelet effect of protamine is not strongly blunted by Aprotinin [16].

Usually, right after the end of CPB, the aPTT normalizes in children between one month and 5 years of age [12], but it could prolong lightly, up to three months of age due to lower levels of circulating XII contact factors, prekallikrein and kininogen. Only three patients in each group were in this age group. However, the aPTT measured 4h after protamine administration was prolonged significantly in the Aprotinin group, and one should surmise that in this group the therapeutic effect of Aprotinin (which prolongs the aPTT), or prolongation of aPTT was caused by waste and/or rebound effect of heparin, whose action is enhanced by Aprotinin [24]. It was reported recently by Nguyen et al. [25] in 2008, that the urinary levels of activity of Aprotinin 2 hours after start of CPB reached 434.2 ± 52 UIC/ml in children, in doses similar to those used herein. Despotis et al. [24] in 1996, showed that Aprotinin does not prolong the PT. Thus, the slight and transient increase in INR in both groups, 4 hours after protaminization, indicates that the consumption of coagulation factors during CPB has not been entirely avoided by the drug.

A limitation of this study is the small number of cases for comparison between groups, with the increase of desirable and necessary sampling, but currently hampered by the removal of Aprotinin in the market. Thus, we used the most powerful test in the sample, compared with the non-parametric test. Statistical analysis through the model above mentioned in the methodology (multiple regression) allowed to demonstrate that the sample is viable and reliable for analysis. The risk is of not being detected a possible significance between the groups that may exist if there may be a larger sample, as the difference in volume of bleeding. The error that can occur is of type I. Thus, under these considerations, we observed in this study that Aprotinin had no significant effect on postoperative bleeding of these acyanotic children undergoing correction of congenital heart disease using CPB, but led to an interesting quantitative preservation of platelets, which may be the focus of future researches with larger series.

CONCLUSIONS

Aprotinin quantitatively preserved platelets, but did not affected postoperative bleeding sgnificantly in these children undergone surgery for congenital heart defect correction.

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