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Hemolysis in extracorporeal circulation: relationship between time and procedures

Hemólise na circulação extracorpórea: correlação com tempo e procedimentos realizados

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Abstract

Introduction: **Extracorporeal circulation (EC) is very important in cardiac surgery but causes significant damage to the blood, including hemolysis.**

Objective: **To quantify the rate of hemolysis at different times during EC in elective coronary artery bypass grafting.**

Methods: **We measured rates of hemolysis of 22 patients at 6 different times during myocardial revascularization during EC: T0 - before the start of EC, T1 - five minutes after of the EC initiation, T2 - 30 minutes of EC, T3 - immediately before the aortic unclamping, T4 - immediately before passage of the residual volume to the patient and T5 - five minutes after the passage of the residual volume to the patient. Rates of hemolysis were calculated between the intervals of time: T0-T1; T1-T2; T2-T3; T3-T4 and T4-T5.**

Results: **The first 5 minutes after the EC showed the highest rate of hemolysis (*P* = 0.0003) compared to the others calculated rates, representing 29% of the total haemolysis until T4 (Immediately before passage of the residual volume to the patient).**

Conclusion: **There were no significant changes in the rate of hemolysis during the suction in the aortic root (*P* > 0.38), nor with the procedure used for the passage of the residual volume of blood in the circuit to the patient.**

Keywords: **Extracorporeal circulation. Hemolysis. Blood.**

Resumo

Introdução: **A circulação extracorpórea (CEC) é indispensável para a maioria das operações cardíacas, mas causa danos significantes ao sangue, dentre eles a hemólise.**

Objetivo: **Quantificar as taxas de hemólise em diferentes tempos nas operações para revascularização do miocárdio com uso de CEC.**

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Abbreviations, acronyms and symbols	
CPB	Cardiopulmonary bypass
HLp	Free plasma hemoglobin
Ht	Hematocrit
PVC	Polyvinyl chloride
RPM	Rates per minute
Tx	Hemolysis rate
TxG	Overall hemolysis rate

Métodos: **Foram medidas as taxas de hemólise de 22 pacientes em 6 tempos distintos durante a revascularização do miocárdio com uso de CEC: T0 - antes do início da CEC, T1 - 5 minutos após o início da CEC, T2 - com 30 minutos de CEC, T3 - imediatamente antes do despinçamento da aorta, T4 - imediatamente antes da passagem do volume residual para o paciente e T5 - 5 minutos após o término da passagem do volume residual para o paciente. Foram calculadas as taxas de hemólise entre os intervalos de tempo: T0-T1; T1-T2; T2-T3; T3-T4 e T4-T5.**

Resultados: **Os primeiros 5 minutos após a CEC demonstraram maior taxa de hemólise (*P* = 0,0003) em comparação às outras taxas calculadas, representando 29% da hemólise total até T4 (imediatamente antes da passagem do volume residual para o paciente).**

Conclusão: **Não foram observadas variações significantes nas taxas de hemólise durante a aspiração na raiz da aorta (*P* > 0,38) nem com o procedimento utilizado para a passagem do volume residual de sangue no circuito para os pacientes.**

Descritores: **Circulação extracorpórea. Hemólise. Sangue.**

INTRODUCTION

Cardiopulmonary bypass (CPB) is the technology that enabled the great advance in cardiac surgery. Although it is an indispensable technique for correction of most heart disease requiring surgical correction, still carries intrinsic features that promote damage to the body. Among these changes we highlight the damage to the blood cells, particularly hemolysis.

Hemolysis is found in all surgical procedures using extracorporeal circuits, as shown in several studies that identify increasing levels of free hemoglobin in plasma and decreased levels of haptoglobin during and after CPB [1].

Hemolysis can occur in three ways: by natural selection of the spleen, physico-chemical imbalance (usually pathological), or by exposing the cells to a non-physiological mechanical stress [2].

In the case of CPB, hemolysis occurs mechanically, either by direct trauma effect of the passage of blood through the rollers or by exposure to different surfaces at different speeds.

The hemolytic aspects have been studied by several researchers in an attempt to isolate and understand the factors causing hemolysis [2-5]. Experimentally, the red blood cells can be damaged during the flow of two factors acting simultaneously: the level of shear stress and exposure time of the cell to such stress [2,6,7]. In flows with high shear stress and exposure time relatively low, it is expected low level of hemolysis. Moreover, we found high hemolysis rate when flow presenting low shear stress, but sufficiently long exposure time. Therefore, one of the basic requirements for developing heart pump is a great compromise between shear stress and exposure time.

In CPB pump two rollers rotate within a raceway (pocket) compressing a flexible tube to promote movement of fluid (blood). The adjustment of the roller pumps is an important factor in hemolysis rates. Calibration is the technique of adjusting the distance between the roller and raceway (occlusion) and is intended to determine the point where the roller only internally collapses the tube without compressing its walls.

The static method or measure of drop rate is the most used in Brazil [8] and consists of observing a falling liquid column, representing the resistance against which the pump will work. Conventionally, the occlusion is adjusted to allow a falling speed of 2.5 cm/min from a column of about 1.000 mm of saline solution. However, few professionals actually use the methodology recommended in the literature [6]. This technique also presents operational difficulties for use in the operating room combined with the lack of reproducibility when performed with silicone tubes [3,9].

Hemodilution is desirable in CPB and hematocrit between 20% and 30% are accepted as suitable for maintenance of oxygen supply to tissues and promote protective effect, preventing contact between erythrocyte and lysis when passing through the rollers.

Studies show that aspiration during procedures involving CPB is responsible for severe hemolysis [10,11] and that the negative pressure and blood exposure to the air, when acting alone, produced no hemolysis, but with the combination of the two factors [12].

The peculiarities of each service in CPB procedures may also influence the rates of hemolysis (suction on the aortic root to prevent air embolism and the passage of residual volume from the CPB system for the patient).

The aim of this study was to quantify the rates of hemolysis at different times associated with CPB procedures in CABG procedures.

METHODS

This study measured the degree of hemolysis during CPB surgeries for CABG procedures in a public hospital in the state of Sao Paulo - Brazil. This study was previously submitted to the Ethics Committee of the Institution, and was approved under protocol 0749.0.146.000-08.

We assessed 22 adult patients from March to August 2010. The study included patients older than 18 years, irrespective of the gender, undergoing isolated CABG. We excluded patients with cognitive problems, pregnant patients and patients who refused to sign the informed consent.

Was used to drive the blood a "Bakey" type roller pump (an arterial pump module, two aspirator pump modules and a blood cardioplegia pump module) and oxygenation was performed with the aid of a membrane oxygenator, all manufactured by Braile Biomédica Ltda.

The tubing used in all surgery was polyvinyl chloride (PVC) with 3/8 inch arterial inner diameter and 1/2 inch inner diameter for venous drainage and 1/4 inch inner diameter for aspirators. Silicone tube with 1/2 inch inner diameter was used for moving the blood through the roller pumps. In the CPB circuit was used oxygenator venous reservoir filter with capable of filtering particles larger than 100 micrometers, and in the arterial line, a filter of 40 micrometers, manufactured by Braile Biomédica.

Washing of the extracorporeal circuit was performed using ringer lactate solution, which was discarded after the effective circulation, replaced by new volume of the same solution as standard technique.

The formation of prime took into account the prior patient's hematocrit value, calculating hemodilution between 25% and 30%. The prime consisted of ringer lactate with albumin or mixed (ringer lactate + albumin + red blood cells), when the calculations demonstrate hemodilution hematocrit less than desired.

At the end of CPB, the residual volume of the oxygenator and arterial circuit was transferred to the venous reservoir, by inversion of the raceway (pocket) tubing of the arterial roller pump and infused into the phlebotomy of the saphenous vein, which was catheterized after its removal in the surgical field. For this, we used the cardioplegia circuit with prior ligation performed using the venous reservoir tubing outlet. Blood was aspirated from the venous reservoir and pumped by roller cardioplegia, through the cardioplegia reservoir until the patient's venous network. This infusion flow did not exceed 200 ml/min.

The roller pump was adjusted as follows: the pump circuit has been filled, performed removal of air, removal of volume and replacement in order to form prime. With the pump stopped, the arterial line and the cardioplegia line were clamped. It was maintained open only recirculation line which communicates the oxygenation chamber with the venous reservoir, which has a length of approximately 50 cm. The roller was positioned at the point of maximum occlusion, vertically to the center of the raceway. The adjustment is accomplished by permitting the liquid in drop speed of 15 cm/min through the recirculation line. The procedure was repeated for roller B.

During the procedure, 6 blood samples were collected of 3 ml each, at the following times: T0 - before the start of CPB, T1 - 5 minutes after the start of CPB, T2 - with 30 minutes of CPB, T3 - immediately before the aortic unclamping, T4 - immediately prior to passing the residual volume for the patient and T5 - 5 minutes after the passing of the residual volume to the patient.

During the collections were recorded values of hematocrit, hemoglobin, nasopharyngeal temperature, blood pressure and higher blood pump rotation.

Free hemoglobin in plasma (FHp) was calculated by the Drabkin & Austin method [13] with the aid of a spectrophotometer Bioplus 200F (Bioplus, São Paulo, Brazil).

The hematocrit (Ht) was measured simultaneously with the withdrawal of blood samples for blood gas analysis (Radiometer ABL3, Copenhagen) and data concerning the FHp measurement at time t have been "corrected" for hemodilution according to the formula:

$$HLp = \frac{HLp(t) \cdot Ht_{base}}{Ht(t)}$$

Equation 1

The Htbasic value was considered the average hematocrit of all patients (27.8%).

Hemolysis (Tx) rates were calculated for the time intervals listed below:

$$Tx1 = \frac{HLp1 - HLp0}{T1 - T0}$$

Equation 2. First 5 minutes of CPB

$$Tx2 = \frac{HLp2 - HLp1}{T2 - T1}$$

Equation 3. 25 minutes after CPB

$$Tx3 = \frac{HLp3 - HLp2}{T3 - T2}$$

Equation 4. Elapsed time for procedures

$$Tx4 = \frac{HLp4 - HLp3}{T4 - T3}$$

Equation 5. Aspiration in the aortic root (intensive aspiration at the time of release of the aortic clamp)

$$Tx5 = \frac{HLp5 - HLp4}{T5 - T4}$$

Equation 6. Passing of residual volume (roller pump maneuver)

$$TxG = \frac{HLp4 - HLp0}{T4 - T0}$$

Equation 7. Global rate of hemolysis (CPB)

$$TxG_1 = \frac{HLp4 - HLp0}{T4 - T0}$$

Equation 8. Global rate of hemolysis in patients receiving packed red blood cells (n = 8).

$$TxG_2 = \frac{HLp4 - HLp0}{T4 - T0}$$

Equation 9. Global rate of hemolysis patients not receiving packed red blood cells (n = 14).

Patients receiving concentrated red blood cells during surgical procedures were recorded, as well as the storage time of the concentrate in the blood bank.

Statistical analysis

We used the Shapiro-Wilk test for normality of the data verification and analysis of variance was calculated using the Kruskal-Wallis test. The difference between groups was calculated by the Student-Newman-Keuls test. The Mann-Whitney test was used to compare means. In all assessments, a P value <0.05 was considered statistically significant.

RESULTS

Table 1 shows the demographic data of 22 patients, mean CPB time, mean body temperature and hematocrit during surgery.

Table 2 lists the average of six times measured during CPB, the respective values of free hemoglobin in the plasma and the rates of hemolysis.

Eight patients received packed red blood cells during surgery with stay time in the blood bank before the use of 1.1 ± 0.4 days. Each patient received only a bag of concentrate with 338 ± 22 ml. The overall hemolysis rate of patients who received packed red cells (TxG1) was 0.6 ± 0.2 (mg/dl/min). In fourteen patients who had not received packed red blood cells, the overall rate of hemolysis (TxG2) was 0.6 ± 0.3 (mg/dl/min). There were no statistical differences between groups ($P > 0.82$).

Table 1. Demographic data of 22 patients and cardiopulmonary bypass time, mean body temperature and hematocrit. Values are expressed as mean and standard deviation.

Age (years)	60 ± 9
Body area (m2)	1.8 ± 0.2
Gender (M/F)	11/11
CPB time (min)	83.5 ± 23.5
Temperature(°C)	35.3 ± 0.4
Hematocrit (%)	27.8 ± 2.7

Table 2. Times recorded during CPB with respective measures of plasma free hemoglobin and hemolysis rates. Values listed as mean ± standard deviation.

Time(min)					
T0	T1	T2	T3	T4	T5
0 ± 0	5.0 ± 0.3	30.5 ± 2.4	64.5 ± 24.3	86.0 ± 31.2	97.0 ± 20.9
HLp (mg/dl)					
HLp0	HLp1	HLp2	HLp3	HLp4	HLp5
8.2 ± 2.5	23.2 ± 7.2	32.8 ± 10.2	48.5 ± 20.4	58.4 ± 23.2	51.5 ± 19.2
Hemolysis rate (mg/dl/min)					
Tx1	Tx2	Tx3	Tx4	Tx5	TxG
2.9 ± 1.7	0.4 ± 0.4	0.4 ± 0.5	0.6 ± 0.8	– 0.7 ± 1.0	0.6 ± 0.2

Table 3 shows the comparison between the average rates of hemolysis (ANOVA) among groups with respective probability values (*P*-value). Data were not normally distributed ($P < 0.05$).

Table 3. Comparisons hemolysis rates between groups: Tx1, Tx2, Tx3, Tx4 e Tx5.

Groups	*P-value*
Tx1 and Tx2	< 0.0001
Tx1 and Tx3	< 0.0001
Tx1 and Tx4	0.0003
Tx1 and Tx5	< 0.0001
Tx2 and Tx3	0.78
Tx2 and Tx4	0.38
Tx2 and Tx5	0.0003
Tx3 and Tx4	0.55
Tx3 and Tx5	< 0.0001
Tx4 and Tx5	< 0.0001

DISCUSSION

It is well established that surgical procedures using CPB promote damage to the blood cells, especially red blood cells by identifying increasing levels of free hemoglobin in plasma and decreased levels of haptoglobin during and after CPB [1].

Cardiac surgeries using CPB exposes blood to a request physical importantly, promoting the destruction of blood cells, particularly hemolysis.

Hemolysis occurs by several factors, which may or may not be associated: roller pump on excessive occlusion [14], shear stress [2,6,7,15], blood-air interface and negative pressure [12] and artificial surfaces [14].

This study aims to identify the rates of hemolysis in surgeries for CABG with CPB using the static calibration method adapted to the conditions of the service. This adaptation was performed by the operational limitation of its use in the operating room and because it is not a technique with repeatability when used with silicone tubes as demonstrated in recent studies [3,9]. We also tried to identify the influence of using the aspirator in a more intense manner (at the time of aortic unclamping, in order to remove air from the left ventricular chamber) and the passage of the residual volume of the CPB circuit, hemolysis rates.

The Tx1 rate of hemolysis was calculated considering the evolution of hemolysis during the first 5 minutes of CPB and was the highest rate among the measured intervals (Table 3).

During CPB, hemodilution is performed with the help of the venous reservoir where the prime is stored. Simultaneously, the blood begins to be drained into the venous reservoir and prime is infused into the patient by the motion of arterial pump, until there is a complete mixture of prime and blood. This initial procedure provides a small fraction of time in which blood passes through the rollers without being diluted. This procedure favors the breaking of red blood cells. Another important consideration is that some erythrocytes, already weakened or aged and with less flexible membranes, when requested by the mechanical action of the rollers are broken, resulting in further increasing rates of hemolysis.

Several studies have demonstrated the great influence of the use of blood aspirators in hemolysis [10-12], being considered by some the greatest cause of destruction of red cells when performed simultaneously air-blood. In an article published in 1958, McCaughan et al. [16] demonstrated that aspiration of air mixed with blood contributed to increased levels of hemolysis compared to intermittent suction blood without this interface. Pohlmann et al. [12] demonstrated in an in vitro study that hemolysis is not caused by exposure to air or negative pressure alone, but by combining these factors. The increased hemolysis is directly related with the increase of negative pressure applied to the gas-blood interface.

In a recent study, Vieira et al. [17] studying the wall thickness of the silicone tube used in the pocket of the rollers, found that greater wall thickness promotes greater suction force and consequently higher pressures of aspiration and tubes of smaller thickness had flow limitation from 60 revolutions per minute (RPM). In this case, hemolysis may occur in two situations described above. The first by over-rotation, allowing for greater mechanical trauma caused by higher rotation without increasing its flow. The second case is the high pressure levels provided by a greater wall thickness, which leads to more pronounced shear stresses.

Tx2, Tx3 and Tx4 rates showed no differences in the hemolysis rates. There was an expectation that Tx4 associated with the intensive use of aspirator, had a higher rate of hemolysis compared to the Tx2 Tx3 rates, but this was not observed.

One hypothesis for the equality of Tx4 rates compared to the Tx2 Tx3 rates was that measures of Tx4 were predominantly characterized by continuous suction of the aortic root. The tube was almost totally submerged during suction and air-blood interface was reduced. This explanation is supported by the results obtained by Pohlmann et al. [12] however, independently the thickness of the tubes in the aspirator pockets was not were not controlled, we expected greater hemolysis by the intensity use of suction, which was not found.

Another point of interest of the study was to assess the influence of the passage of the residual volume of blood in hemolysis. The TX5 rate was calculated between the instants: immediately prior to passing the residual volume for the patient (T4) and 5 minutes after the passing of the residual volume for the patient (T5).

The TX5 rate measured was negative (-0.7 ± 1.0) over the time interval between T4 and T5, in the mean calculated of the 23 patients of 16.3 ± 3.3 minutes (mean $\pm$ standard deviation). In between, there was recovery in rates of hemolysis performed by the body. The results indicated no increase in hemolysis rates as a consequence of the procedure for passing residual volume.

Some authors emphasize that transfusion of red cells stored for longer than 2 weeks is associated with increased postoperative complications and reduced short-term survival and long-term after heart surgery [18]. Other authors point out that the storage time of red blood cells is not a risk factor for early and late mortality in patients undergoing coronary artery bypass grafting [19].

In our study, the group receiving packed red blood cells between one and two days of storage in blood bank (n=8) and the group that received packed red blood cells (n=14) showed no differences ($P > 0.70$) in overall hemolysis rates (TXG).

CONCLUSION

The first 5 minutes of CPB demonstrated a higher rate of hemolysis ($P = 0.0003$) and accounted for 29% of total hemolysis measured until the passage of residual volume for patients.

The suction on the aortic root to prevent air embolism showed no significant variations in the rates of hemolysis ($P > 0.38$).

There were no differences in hemolysis rates with the procedure used for passing residual volume of blood in the circuit for patients.

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