Platelet Functions in Cardiopulmonary Bypass Surgery

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Abstract

Background- Haemorrhage after Cardio Pulmonary Bypass (CPB) Surgery is a well recognised complication that leads to significant morbidity and mortality. The incidence varies between 5-25% depending upon the clinical situation. Several factors are implicated as causative but none have been precisely proved.

Methods- Our study was an attempt to evaluate the haemostatic defect with particular reference to platelet function abnormalities during cardio pulmonary bypass surgery, in order to reduce the morbidity and mortality associated with post CPB haemorrhage. Flow cytometric evaluation of different platelet glycoproteins like GPIb/IX, GPIIb/IIIa and GMP-140 was done.

Results: The marker expression showed deregulation during surgery which returned to base after bypass was terminated. In contrast, the cases with bleeding showed significant variation. P-Selectin (GMP 140) expression decreased progressively till 3rd post-operative day showing lack of activation of platelets in cases of severe bleeding.

Conclusion- Longer duration of CPB initiates plasmin generation through heparin, which raises the PAI-1-tPA complex and thereby down regulating the functions of platelets. This suggests a link between duration of CPB, bleeding, platelet dysfunction and fibrinolysis. Hence serial estimations of the levels of GMP-140 and tPA can predict severe bleeding.

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Key Words: CardioPulmonary Bypass; Platelet dysfunction; flowcytometry; platelet glycoproteins; haemorrhage

Introduction

Haemorrhage after Cardio Pulmonary Bypass (CPB) Surgery is a well recognised complication that leads to significant morbidity and mortality. The incidence varies between 5-25% depending upon the clinical situation. Several factors are implicated as causative but none singularly has been precisely proved. Amongst these, platelet dysfunction seems to be the most plausible explanation for coagulopathy after CPB.

Although CPB surgery is now viewed as a routine procedure, there is still significant morbidity and mortality related to and initiated by excessive haemostatic activation. Further research and optimisation of therapeutic approaches to control haemostatic function during and following bypass procedure is required. This study is an attempt to understand the pathophysiology and evaluate various causes of post CPB haemorrhage in the Indian setting in order to reduce the morbidity and mortality as well as to reduce the requirement of blood and blood product transfusion in such cases.

Material and Methods

60 consecutive cases undergoing cardio pulmonary bypass surgery at a Cardio Thoracic Centre from Aug 1997 to Nov 1999 were included in this study. Exclusion criteria

included aspirin intake 3 days prior to surgery, cases of known bleeding disorder, poor Left Ventricle ejection fraction and concomitant renal/hepatic dysfunction.

On the day before surgery the complete clinical data case was collected. Blood was collected for baseline investigations like Hb, PCV, TLC, DLC, platelet count, Blood group, PT, PTTK, TT and fibrinogen level.

On the day of surgery and postoperative period the venous blood samples were collected and labelled as 30 mins before starting CPB and anaesthesia (pre), 20-30 mins after starting CPB (during), 60 mins after termination of CPB (post op) and 1st post operative day after CPB(D₁).

10 ml of venous blood was collected in plastic tubes with 3.8% Sodium Citrate as anti coagulant in the ratio of 1:9 (citrate: blood) volumes with minimal tourniquet pressure and 2 ml in EDTA vial for CBC. Platelet Rich Plasma (PRP) was prepared by centrifugation of the samples at 100-150g for 10 mins at 22°C. Each sample was processed for Flow Cytometry of platelet glycoproteins.

On the day after surgery each case was reviewed with reference [a] duration of CPB, [b] drugs taken during CPB- Aprotinin (given in the dose of 5,00,000 IU, single dose IV bolus, during surgery in high risk cases), [c] Heparin-LMWH/UFH, [d] Protamine sulphate required for reversal, [e] outcome of CPB, [f] complications if any noticed, [g] amount of blood loss (12 hours postoperatively, measured by chest tube drain

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collection) [h] temperature ($^{\circ}\text{C}$) maintained during CPB and [j] ACT values before, during and after surgery.

The same parameters were checked on the first and third postoperative day or till platelet function returned to normal. Samples were analysed till the third post operative day in the initial few cases and we found the platelet function returning to baseline and hence sampling omitted after the first postoperative day.

Flow cytometry (Reagents and specimens) included Platelet Rich Plasma (PRP), Monoclonal antibodies, 0.4% para formaldehyde, Phosphate buffered saline [PBS]

Equipment was Flow cytometer -Coulter EPICS-XL (UK)

Method

1. 50 μl of PRP was collected directly into each of the plastic tube for the required number of monoclonal antibodies to be tested for that particular sample.
2. 5 μl of the labeled (FITC) monoclonal antibody was put into each of the PRP sample tubes and incubated in the dark for 30 minutes.
3. At 30 minutes the reaction was stopped by adding 200 μl of 0.4% para formaldehyde into each of the test tube and the test tube sealed with polyfilm to prevent oxidation and evaporation loss before analysis.
4. The sealed tubes were kept at $4-8^{\circ}\text{C}$ pending analysis.
5. A similar test tube containing 50 μl of a normal control PRP was taken and 5 μl of negative control, i.e. FITC labelled IgG was also similarly incubated for 30 minutes in the dark, after which 200 μl para formaldehyde was added and kept sealed at $4-8^{\circ}\text{C}$ for analysis.
6. On the day of analysis, 200 μl of PBS was added to each sample tube. Mixed by vortexing and analyzed in the flow cytometer using the specific protocol. The percentage of cells expressing the antigen was analyzed against the negative control for the day.

Results were recorded as percentage expression of fluorescence. The blood samples obtained as pre, during, post operative and next day post op (D_1) samples were processed and analysed for each case. Results were analysed using Microsoft Excel 2000 computer software utilizing the appropriate statistical tests.

Results

Clinical findings

The present study was carried out in 60 cases (31 females and 29 males). The mean age + SD was $30+20.71$ years. Pre-operative symptoms included dyspnoea on exertion and palpitation in the adults and repeated respiratory tract infections in the young (< 18 years). History of embolic phenomenon was present in one case.

25 had congenital disorders (Ventricular Septal Defect-6, Atrial Septal defect-16 and Tetralogy of Fallot-3), 11 had coronary artery disease, 22 valvular heart disease and 2 Left Atrial Myxoma. The duration of CPB varied from 20 to 241 minutes, with mean + SD value of $146+52$ minutes. The mean

value of Heparin dose was 290 mg (range 55-1000 mg) with standard deviation (SD) of 52mg. All the cases were reversed from Heparin using Protamine sulphate. The mean (+SD) protamine sulphate dose administered was $789 + 590$ mg. It was observed that maximum blood loss was in the case of CABG and valve replacement (AVR) and minimum in ASD and VSD repair. The requirement of blood varied between nil to 5 units with the mean + SD being $2.1 + 1.1$ units and use of components varied between 0 to 4 units of platelet concentrate (PC), 0 to 4 units of cryoprecipitate and Fresh Frozen Plasma (FFP) respectively in the entire group.

Blood loss / bleeding: The postoperative blood loss (upto 12 hours post op) varied between 100 ml and 1520 ml with the mean + SD of $425.08+280.14$ ml ($n=60$) The amount of postoperative blood loss was directly related to the duration of CPB as analyzed by regression analysis and has shown statistically significant correlation (Fig 1). 33 patients had minimal bleeding (less than 400 ml blood loss), 23 had moderate blood loss (between 400 and 1000 ml), 4 had severe blood loss (more than 1000 ml). Duration of CPB which showed statistically significant correlation (Table 1) The bleeding episodes has shown definite pattern of platelet glycoprotein expression on flow cytometry like persistent fall of GMP-140 value in cases of severe bleeding as compared to recovery of the activation marker in those with minimal to moderate bleed (Fig 2). 20 cases were operated under Hypothermia (Temp $< 32^{\circ}\text{C}$) and 40 were operated at normothermia (Temp $32 - 37^{\circ}\text{C}$). There was significant variation in the haematological/coagulation parameters and platelet glycoprotein receptor expression in relation to temperature. Aprotinin was used in selected 8 cases in the dose of 5,00,000 IU (70mg) during surgery. Our study revealed significant difference ($p < 0.005$) in the requirement of blood

Fig 1 : Showing the result of regression analysis of post operative blood loss (12 hours post op, chest tube drain collection) against CPB duration (p value < 0.005) ($\rightarrow$) shows one case operated without CPB-beating heart surgery. The three-outlier cases are the ones who had valve repair surgery.

Table 1**Bleeding in relation to different parameters (mean + SD)**

Bleeding type	Amt of bleed (ml)	Age (yrs)	Duration of CPB (min)	Heparin (mg)	Protamine (mg)	Temp °C
A[n=33]	<400	<24.85±19.4	80.36±42.08	<224.6±125.9	>620±386.6	33.9±2.9
B[n=23]	400-1000	36.61±20.11	82.39±52.96	361.17±233.9	876.4±539.9	34.4±2.5
C[n=4]	>1000	43.25±24.42	119.5±59.6	400±215.05	1587.5±1135.3	31.3±4.1
A vs. C		NS	<0.005	NS	NS	NS
A vs. B		NS	NS	NS	NS	NS

Not significant (NS)

Table 2**P-Selectin expression (%) (mean + SD) during CPB in cases of Bleeding**

Amount of bleeding	Pre	Op	Post op	D1	Preop Vs. Post op	Preop Vs. D1
Mild	9.41±7.29	6.47±4.91	6.28±6.02	8.11±7.78	NS	NS
Moderate	12.13±10.01	10.39±14.98	10.18±10.62	10.57±11.19	<i>p</i> <0.01	<i>p</i> <0.05

and blood components and in the expression of platelet glycoprotein GP Ib/IX (*p* < 0.005) between patients with and without Aprotinin.

Flow cytometry

The platelet glycoproteins were analyzed on Coulter EPICS-XL (UK) flow cytometer with FITC conjugated monoclonal antibodies to GPIb/IX, GP IIb/IIIa and GMP140 (Becton Dickinson/Pharmingen, USA). The expression of platelet glycoproteins GPIb/IX, GP IIb/IIIa and GMP140 or P-Selectin has showed significant variation in different clinical settings (Table 2). The percentage expression in all the uncomplicated cases showed that the degree of expression decreases during surgery/CPB and recover fully to the basal pre operative level by the first postoperative day. The cases of severe bleeding had significant variation in the expression of P-Selectin, which progressively fell during the postoperative period (Fig 2). P- Selectin levels in cases with severe bleeding were much lower as compared to moderate

and mild bleeding even at the pre operative stage, a factor which has predictive value for haemorrhage. Platelet GP Ib/IX expression had significant change in relation to CPB duration (*p* <0.05). CPB of more than 150 minutes showed very low expression of GPIb/IX during and immediately after operation.

Complete blood count and coagulation tests

The mean haemoglobin level was 12.2 gm% (range 16-08 gm%) before surgery, falling to a mean value of 10 gm% on the day after surgery and the mean platelet count which was $1.9 \times 10^9/L$ before surgery fell to $1.6 \times 10^9/L$ after surgery and showed recovery to a mean value of $1.71 \times 10^9/cmm$ by the 3rd post operative day. The haematological and coagulation parameters in cases of post CPB bleeding are shown in Table 3. The duration of CPB also shows significant alteration of the coagulation parameters like platelet count, PT and PTTK. But tPA estimation revealed failure of levels to return to baseline in cases with severe bleeding when compared to uncomplicated cases (Fig 3). Cases of CABG showed consistent fall in levels of fibrinogen throughout surgery while in cases of valvular heart disease, the fall in levels were more gradual and had early recovery to baseline levels after termination of CPB. The intra-operative ACT level was maximum in cyanotic heart disease and minimum in CABG while the fall in pH was maximum intra-operatively in CABG and cyanotic heart diseases showed delayed recovery of pH after termination of CPB.

Outcome of surgery

Of the 60 cases, 57 were discharged home after a mean hospital stay of 8 days. Complications developed in 8 cases (13.3 %) with Bleeding (>1000 ml) in 3 cases (5%), hemi paresis in one and poor Left Ventricular (LV) function in 4. 3 cases expired (5%) within 6 hours of surgery. All the fatal cases had intractable left ventricular dysfunction. 3 cases (5%) developed severe bleeding (> 1000 ml blood loss within 12 hours of Post operative period) which was controlled with blood component therapy using mostly packed cells and platelet concentrates. All bleeding cases belonged to the valvular heart surgery group. Aprotinin was not administered

Fig 2 : Showing the variation of platelet GMP 140 expression in different degrees of post operative bleed. Note the lack of return of the GMP 140 levels in severe bleeding cases after termination of CPB (*p* value <0.05)

Table 3**Haematological parameters and CPB temperature (Mean +SD)**

	HbGm%		Platelet count x 10 ⁶ /L		PT(Sec)		PTTK(Sec)	
	Before**	After**	Before**	After**	Before**	After**	Before**	After**
Temp <32°C*	12.9+1.3	11.05+1.02	193.3+27.9	176.2+19.3	15.91+1.32	17.43+2.1	41.30+2.3	48.69+2.7
Temp 32-37°C*	11.8+1.6	10.3+1.4	189.3+28.1	176.3+18.7	15.30+1.56	17.49+1.9	39.83+2.4	45.56+2.3
P value	<0.005*	<0.05**	<0.05*	<0.005**	<0.05**	<0.05**	<0.005*	<0.05**

Fig 3: Showing the marked variation of tPA between uncomplicated case and the cases with severe post operative bleeding.

to any case after they developed bleeding post operatively. Four cases who developed Left ventricular dysfunction were put on Intra Aortic Balloon Pump (IABP) support, out of which only one could be salvaged. None of the cases had heparin-induced thrombocytopenia (HIT).

Discussion

Many factors influence the degree of postoperative blood loss, i.e. the experience of the surgical team, duration of extra corporeal circulation, pre-surgical coagulation status of the patient, prior intake of anti coagulant and antiplatelet drugs and prior treatment with thrombolytic agents. The use of CPB during cardiac surgery is associated with generalised haemorrhagic defect. Although mild, thrombocytopenia and alterations of fibrinolytic and coagulation systems do occur during CPB. Platelet function defect is considered primary for CPB induced haemostatic defect. The nature of platelet function defect remains controversial [2]. Many factors associated with CPB may affect platelet function which include pH, platelet count, hematocrit, drugs, FDP, type of pump priming, type of oxygenation system used and

release of proinflammatory cytokines like IL-6 and IL-1.

The hospital data in the previous three years showed the incidence of postoperative bleeding varying from 9 to 12%, when all cases are taken together. Among the sixty cases studied 3 cases (5%) developed severe bleeding and 3 cases (5%) had fatal outcome, mostly following intractable Left Ventricular dysfunction.

It has been observed [3] that duration of CPB is important in CPB induced hematologic dysfunction. We found that the duration of CPB had no significant relation to CPB temperature i.e. normothermia or hypothermia as observed by other workers [4]. The flowcytometric analysis of the platelets during CPB revealed down regulation of GP Ib/IX expression. Furthermore there was activation of platelets subsequently leading to platelet dysfunction with increasing duration of CPB [4].

The mean blood loss after CPB in adults was reported to be 1000 ml [1]. In the present study the average blood loss was 425.08 ml. We found that there is direct correlation with duration of CPB and amount of postoperative blood loss. In our study we found that the amount of blood loss is also not related to the temperature of CPB contrary to what was reported by other workers [5] who demonstrated increased blood loss with hypothermic CPB. It was observed that Aprotinin given as bolus after CPB produced no benefit, but if given as a continuous infusion resulted in reduction of perioperative and postoperative bleeding and transfusion requirement. It is observed that [6] high dose of Aprotinin is effective in reducing the bleeding during and after CPB. The analysis of our present data revealed that those patients who had excessive bleeding had higher level of heparin as compared to the group who had less bleeding. It has been observed [7] that heparin contributes to haemostatic defect by inhibiting coagulation not only by the effect on anti-thrombin (AT-III) but also by eliciting several adverse effects on platelets and fibrinolytic system.

Different workers reported loss of platelet membrane receptors for both vWF and fibrinogen during and after cardiopulmonary bypass [8]. At the same time, others [9] have demonstrated that CPB does not produce decrease in expression of platelet surface glycoproteins and factors extrinsic to platelets like heparin in

hypothermia and fibrinolysis may contribute to platelet dysfunction. Van Oeveran et al have documented significant preservation of platelet GP Ib/IX receptors with Aprotinin during CPB, resulting in improved haemostasis during and after CPB [10]. However GMP-140 expression did not show significant difference with Aprotinin [6]. During CPB, plasmin produces platelet activation and cleavage of both vWF and fibrinogen sites, which is responsible for the success of the antiplasmin agent, Aprotinin in reducing post CPB bleed which in turn results from it's ability to protect/preserve GP Ib/IX complex and GP IIb/IIIa complex [11]. They also showed increased activation of platelets during CPB, which comes down after four to six hours of termination of CPB. It has been demonstrated that there is progressive activation of platelets during CPB characterized by granule release affecting upto one third of all circulating platelets. GMP-140 expression is correlated with platelet clearance indicating that under normal circumstances, activated platelets do not remain in the circulation but are actively cleared [12]. It has been documented [2] that CPB did not result in decrease in platelet surface expression of GP Ib/IX complex or GP IIb/IIIa complex and the overall P- Selectin expression on the surface of platelets was minimal. CPB resulted in no significant increase in platelet surface expression of GMP-140 [13].

In our study, we found that there was a sustained reduction of GP Ib/IX expression over the platelets during surgery, which recovered to baseline by day one after CPB. GMP-140 expression also had similar reduction during after surgery. Pre operatively the GMP-140 level was high in the LA Myxoma and in other groups the values were around 10%. This indicates that in some conditions the activated platelets circulate preoperatively. This emphasizes the need to assess platelet surface glycoproteins preoperatively by flowcytometry. There was significant change in the percentage expression of GP Ib/IX in cases with Aprotinin and in hypothermic patients during CPB. In cases of bleeding more than 1000 ml on day one after CPB, we found that the GMP 140 expression failed to return to baseline after termination of CPB (Fig. 4).

It has been documented that depletion of leukocytes and platelets during operation prevents the deterioration of cardiac function after cardiac operations using CPB [14]. In the present study, out of the three cases that developed left ventricular dysfunction, two had high levels of expression of P-Selectin. Therefore, it can be said that cytokines have a role in producing myocardial dysfunction. In short it can be said that the above study establishes the role of platelets in producing post CPB bleeding and explains its pathophysiology.

Fig 4 : Showing GMP-140 expression over platelets in different groups of patients. Note the significant change of expression of the receptors in the post operative samples in CABG and Valvular surgery cases and the failure of the receptor levels to return to base line in valvular surgery cases even on the D1 samples (the group which had severe bleeding).

In conclusion, flow cytometric evaluation revealed reversible down regulation of the surface glycoproteins and the activation marker during CPB. There was complete recovery of the expression of these glycoprotein receptors within 24 hours of surgery, but GMP-140 expression failed to return after surgery (CPB) in cases of excessive bleeding. Furthermore, the cases with excessive bleeding had reduced expression of GP Ib/IX. Longer duration of CPB initiates plasmin generation through heparin, which raises the PAI-1-tPA complex and thereby activating platelets, manifested by GMP -140 expressions. This suggests a link between duration of CPB, bleeding, platelet activation and fibrinolysis. Monitoring the levels of GMP140 and PAI-1-tPA complex can predict the severity of bleeding.

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