
Original Article***5-Year Incidence of Thrombocytosis and the Effect on Heparin Dose Response and Heparin Requirements***

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ABSTRACT

Thrombocytosis has been shown to be associated with heparin resistance. Contact activation of platelets results in release of Platelet Factor 4 from alpha granules present in the platelet cytoplasm. Platelet Factor 4 is a cationic substance that neutralizes heparin. This could result in inadequate heparinization during cardiopulmonary bypass (CPB). Inability to adequately anticoagulate patients with thrombocytosis could result in a poor clinical outcome. A retrospective review of pump records from 1991 to 1996 was used to assess the frequency of thrombocytosis, describe the demographic characteristics of patients with thrombocytosis, and determine the effects on patient heparin dose response (HDR) and additional heparin requirements. A platelet count of $400,000/\text{mm}^3$ was chosen as a cut-off for thrombocytosis. Of the 3281 patients undergoing CPB during this time period, a total of 571 patients were included in this review: 99 had high platelet counts. The over-all prevalence of thrombocytosis during this time period was 3.0%. Patients with thrombocytosis tended to be younger ($p = .02$), have lower preoperative HCT ($p < .001$), and weigh less ($p < .001$). These patients had lower post-heparin loading dose ACTs, lower HDR, required more additional heparin to reach an ACT of 480 sec before CPB, and required more heparin on CPB to maintain the ACT > 480 sec ($p < .05$). Multiple linear regression was performed and concluded that age, use of NTG and heparin drip preoperatively, and platelet count were significant predictors of the heparin dose response. Use of plasmapheresis to remove platelet-rich plasma (PRP) before CPB was performed in 22 patients, six of whom had high platelet counts. In these patients, removal of PRP resulted in no difference in the amount of additional heparin required pre-CPB to reach an ACT of 480 sec. ($p = \text{NS}$) Additional studies are needed to determine whether use of plasmapheresis is a cost-effective and clinically useful option in patients with thrombocytosis.

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INTRODUCTION

Heparin resistance is a clinical problem frequently faced by perfusionists. It has been reported that 22–45% of patients undergoing CPB fit into this category (1, 2). Heparin resistance has been defined as an increase in the heparin requirements necessary to maintain an activated clotting time (ACT) adequate for CPB (3). Inability to increase the ACT may result in inadequate anticoagulation during CPB, with problems ranging from secondary fibrinolysis, with subsequent utilization of coagulation factors leading to increased postoperative bleeding, to fatal thrombosis (4). Many variables have been related to heparin resistance. A literature search revealed those variables (Table 1) commonly seen in CPB patients.

Thrombocytosis is an increase in the platelet count above normal limits. Platelets contain secretory granules within their cytoplasm: alpha granules, dense granules, and lysosomes. When platelets are introduced to the nonendothelial surfaces of the extracorporeal circuit (ECC), they immediately adhere to that surface and release the contents of these secretory granules (23). The alpha granules in platelets contain several substances that accelerate coagulation, including platelet factor 4 (PF4). PF4 is a cationic substance that potentiates platelet aggregation by inactivating local heparin (23). It can be hypothesized that an increase in the number of platelets present before CPB would result in an increase in the amount of platelet granule contents that would be released upon initiation of CPB. This could result in an increase in the amount of heparin necessary to overcome the procoagulant effects of these granule substances. Thrombocytosis is not thought to be a frequent cause of heparin resistance in cardiac patients, because patients who display this abnormality frequently have some underlying medical disease that precludes heart surgery (24).

Reports of open-heart surgery patients with thrombocytosis have confirmed that heparin resistance is a problem in these patients (15). It is important to determine the correct treatment so that the complications associated with inadequate heparinization are not seen. Several treatments for heparin resistance have been described. It is necessary to recognize that thrombocytosis can be a cause of heparin resistance and to tailor the

treatment to the problem. Of the variables listed in Table 2, only increasing the heparin concentration to overcome the antiheparin effect of the PF4 would be the correct treatment for heparin resistance related to thrombocytosis.

Another option, not previously described in the literature, would be to remove some of the platelets from the patient before initiation of CPB. If the platelets are not present to become activated by the ECC, the associated heparin resistance may not be seen. Plasmapheresis to remove 20–30% of the patient's plasma volume before heparinization is commonly used in CPB patients (29–31). Platelet-rich plasma (PRP) includes the buffy coat that contains the platelets (29).

The purpose of this project was to compute the prevalence of thrombocytosis in the adult population of patients undergoing CPB for cardiac surgery at our institution. Concurrent estimates of the incidence of heparin resistance and heparin requirements are also made to determine the effect of thrombocytosis on these variables. Preliminary data are used to determine the efficacy of PRP removal before CPB on the incidence of heparin resistance and heparin requirements.

MATERIALS AND METHODS

After Institutional Review Board approval for exemption, perfusion records from April 1991–January 1997 were analyzed. Adult patients (age >18 years) undergoing CPB for all procedures at our institution were included in this study. Demographic, laboratory, and operative information (Table 3) were recorded from 3281 patients during this time period.

The normal platelet count at our institution is 150–400,000/mm³. This lab test was drawn the day before surgery on all cardiac patients. Thrombocytosis was defined as a preoperative platelet count >400,000/mm³. All patients with thrombocytosis were included in the study. A systematic sample of the remaining patients was taken to obtain a case/control ratio of 1:4 (32). The resultant sample sizes were 99 cases and 472 controls (total study sample size, 571 patients). Heparin dose response (HDR) was calculated using the formula described by Bull et al (33). Heparin resistance was defined as a heparin dose response < 60 sec/μ/ml. During this study period, plasmapheresis was performed on 22 patients. Platelet-rich plasma (20% of the patients' estimated plasma volume) was removed preheparinization by methods previously described (31). The average concentration of platelets in the product removed at our institution during this time period using this technique was

Table 1: Variables related to heparin resistance (reference #)

Age (5–7)
Coronary artery disease (4)
Heparin drip (8–12)
Nitroglycerin drip (13,14)
Platelet count (7,15,16)
Antithrombin III deficiency (17–20)
IABP use (1,10)
Autologous blood removal (21,21)
Endocarditis (22)

IABP = intra-aortic balloon pump.

Table 2: Treatment for heparin resistance (reference #)

Administer more heparin (25)
Change type of heparin (26)
Administer FFP (2,25,27)
Administer anti-thrombin III concentrate (25,28)

FFP = fresh frozen plasma.

Table 3: Variables

Demographic	Laboratory	Operative
Age	Hematocrit	Volume of PRP removed
Gender	Fibrinogen	Operation
Height	Platelet count	Heparin loading dose
Weight	Baseline ACT	Heparin concentration
Body surface area	Postheparin ACT	Heparin dose response
Blood volume	On-CPB ACT	Additional heparin on CPB
ASA, NTG, heparin drip	Lowest ACT	

ACT = activated clotting time; ASA = aspirin use preoperatively; CPB = cardiopulmonary bypass; NTG = nitroglycerin drip preoperatively; PRP = platelet-rich plasma.

167,500 $\pm$ 63,000/mm³ (30). Descriptive statistics on this subset of patients was also performed.

All patients underwent CPB with mild-to-moderate hypothermia using a membrane oxygenator, either a closed or open venous reservoir, arterial line filter, and 4:1 blood cardioplegia. Anesthetic management was not significantly altered throughout the 5-year time period.

Histograms and normal probability plots were performed on each continuous variable to assess normality. Bartlett's homogeneity of variance test was performed to check for equality of variances. If the variable was normally distributed and the variances were the same for the two groups (cases and controls), pooled *t*-tests were performed to determine differences between the groups. Nominal data were assessed using the chi-square test. Univariate and multivariable linear regression were used to determine which variables affected the presence of heparin resistance after adjusting for relevant confounding variables. A *p* value of .05 was chosen to assess statistical significance. All data, where appropriate, are listed as mean $\pm$ standard deviation.

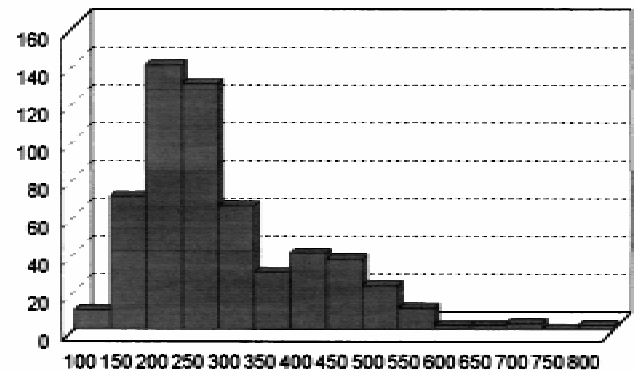
RESULTS

The incidence of thrombocytosis in the 5-year period was 3.02%. The concurrent incidence of heparin resistance was 32%. Figure 1 is a histogram of the platelet counts for the sample. Figures 2 and 3 are histograms of the heparin dose responses obtained on all patients based on pre-CPB data and after initiation of bypass. Figure 3 plots the platelet count against the heparin dose response. The scatterplot is blocked into areas of platelet count/heparin resistance.

Descriptive statistics for the demographic data in each group are shown in Table 4. Patients who had thrombocytosis were significantly younger, weighed less, and had lower preoperative HCT. The type of operation is broken down into study groups in Table 5. Data related to heparin requirements and heparin resistance are shown in Table 6.

The significant univariate predictors of heparin resistance

Platelet count

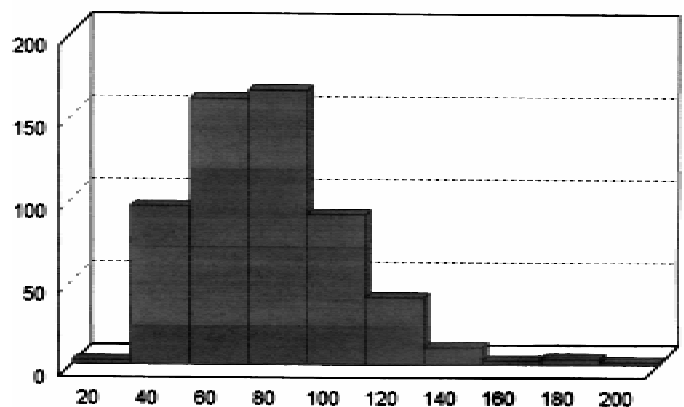


n=571

Figure 1: Histogram of platelet counts for patients in the study. The distribution is slightly right skewed. Abs = absolute, k/cumm = thousand per cubic millimeter.

Heparin Dose Response

Pre-CPB



n=570

Figure 2: Histogram of the heparin dose response obtained pre-CPB for the patients in the study. This variable is normally distributed. Heparin dose response was computed using the formula derived by Bull et al. (33).

were age, platelet count, type of operation, and preoperative heparin drip (*p* < .05). When placed into a multivariable model to control for the interactive effects of the confounders, only age, heparin drip, and platelet count remained significant predictors for heparin resistance (*p* < .01). NTG drip was not a significant univariable predictor; however, when placed into the above model, it also became a significant multivariable predictor (*p* < .01).

The same statistics were computed using the data from the patients who had plasmapheresis. The results are shown in Table 7.

Heparin Dose Response

On CPB

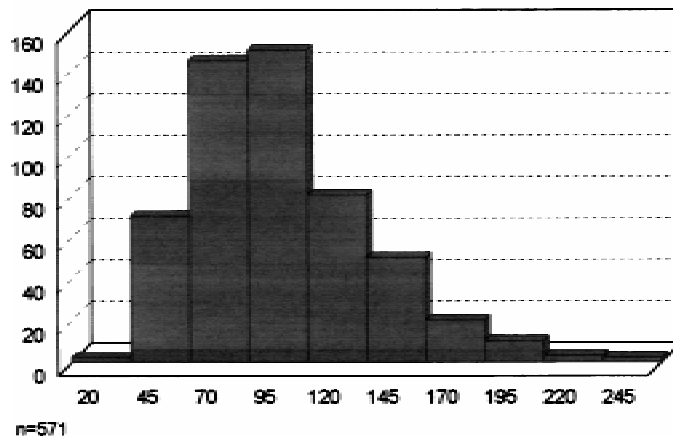


Figure 3: Histogram of the heparin dose response obtained once CPB was initiated. This variable is normally distributed.

Table 4: Results of *t*-tests and chi-square tests on descriptive and preoperative data

Variable	Case	Control	<i>p</i> value
Age (yrs)	59.02 ± 12.9	62.2 ± 11.7	0.025
Gender (% M)	71.7	80.1	0.065
Weight (kg)	76.3 ± 15.2	82.8 ± 16.9	0.01
BSA (m ²)	1.88 ± 0.2	1.96 ± 0.2	0.01
ASA (% yes)	29.3	31.99	NS
NTG (% yes)	29.3	34.3	NS
Heparin (% yes)	15.2	21.2	NS
HCT (%)	36.6 ± 6.1	40.0 ± 5.7	0.01
Fibrinogen (mg %)	437.8 ± 143.5	386.5 ± 103.9	0.057
Platelet count (k/mm ³)	486.4 ± 93.7	236.9 ± 65.1	0.01

ASA = aspirin use preoperatively; BSA = body surface area; Case = high platelet count group; Control = normal platelet count group; HCT = hematocrit; % M = percentage of males; NTG = nitroglycerin drip preoperatively. All values mean ± SD, except for nominal data, which are shown as %.

The prevalence of heparin resistance in patients who had PRP removed did not differ between the two groups, and the univariate predictors of heparin resistance were heparin drip preoperatively, fibrinogen level, and patient weight ($p < .05$). When placed into a multivariable model of heparin resistance, a preoperative heparin drip was the only variable that remained significant ($p = .04$).

DISCUSSION

In vitro work published in 1948 by Conley et al. first determined that the concentration of heparin required to inhibit or delay coagulation is directly related to the number of platelets

Table 5: Distribution of operations performed in each group

Operation	Case	Control
CABG	69	360
Valve	13	62
Heart transplant	1	19
Other	9	10
Combination	7	21
Total	99	472

CABG = coronary artery bypass grafting; valve = aortic, mitral, or tricuspid valve repair or replacement.

present (16). Although several authors list thrombocytosis as a cause of heparin resistance during CPB, there has only been one published case report that actually describes this phenomenon (15). In this report, three patients with platelet counts greater than 600,000/mm³ exhibited extreme heparin resistance. In two patients, additional heparin was required throughout the CPB run to increase the ACT to above the minimal protocol ACT eventually. The third patient died on the operating room table as a result of severe coagulopathy (attributable to secondary fibrinolysis). The first ACT run on CPB in this patient was 195 sec. Examination of the oxygenator post-CPB revealed that the defoamer on the oxygenator was completely clotted. The authors state that the occurrence of thrombocytosis in CPB patients is rare; however, a thorough literature search found no published reports of the prevalence of thrombocytosis.

A multivariable regression analysis on 157 consecutive patients performed by Gravlee et al. in 1987 found platelet count as a significant predictor of heparin resistance. At least one patient in the study did have a platelet count greater than 600,000/mm³, but whether this patient was heparin resistant was not discussed. They conclude that the average increase in the platelet count of 125,000 mm³ will increase the heparin dose requirement by 25% (7).

The first purpose of this project was to compute the prevalence of thrombocytosis in CPB patients. Ninety-nine patients presented with higher than normal platelet counts in a 5 year period at our institution. The resultant prevalence was 3.02%. This value agrees with comments by other authors that the percentage of patients with thrombocytosis is low (15,24); however, in a busy heart program, the number of patients could be large. It is important to recognize that these patients can present with heparin resistance and present a clinical challenge for the perfusionist.

The second purpose of this study was to determine the prevalence of heparin resistance in the same group of patients. Our prevalence of a HDR < 60 sec/μ/ml was 32%. This percentage is similar to published reports; however, the same definition of heparin resistance was not used in any of the reports. Utilization of a consistent definition would result in comparable frequencies between institutions.

Table 6: Results of *t*-tests and chi-square tests on operative data

Variable	Case	Control	<i>p</i> value
Baseline ACT (sec)	128.5 + 20.4	123.5 + 18.7	.023
HLD (units)	24741 + 5663	27119 + 6550	.01
ACT after HLD (sec)	467 + 140	512 + 138	.0041
Heparin concentration (pre-CPB) (μ /ml)	5.0 + 0.8	5.03 + 0.7	NS
HDR (pre-CPB) (sec/ μ /ml)	67.95 + 26.8	77.76 + 26.8	.0012
Heparin resistant (% Yes)	45.4	29.2	.05
Additional heparin pre-CPB (units)	6303 $\pm$ 8995	4038 $\pm$ 7316	.02
Heparin concentration (on-CPB)	4.85 + 1.39	4.53 + 1.16	.033
HDR (on-CPB) (sec/ μ /ml)	98.3 + 39.0	90.6 + 38.4	.074
Additional heparin on CPB (units)	9305 $\pm$ 9147	7486 $\pm$ 8315	.07
Lowest ACT (sec)	407.64 + 72.3	437.9 + 86.7	.02

ACT = activated clotting time; HDR = heparin dose response; HLD = heparin loading dose. All values mean $\pm$ SD, except for nominal data, which are shown as %.

Table 7: Results of *t*-tests and chi-square tests on descriptive and operative data for 22 patients with plasmapheresis pre-CPB

Variable	Case	Control	<i>p</i> value
<i>N</i>	6	16	
Age (yrs)	59.3 $\pm$ 11.7	61.4 $\pm$ 9.64	NS
Platelet count (k/mm ³)	536 $\pm$ 107	262.6 $\pm$ 69.4	0.0012
Baseline ACT (sec)	121.2 $\pm$ 26.8	120.9 $\pm$ 16	NS
HLD (units)	25500 $\pm$ 6285	26569 $\pm$ 5034	NS
ACT after HLD (sec)	450 $\pm$ 100	570 $\pm$ 182	0.067
Heparin concentration (pre-CPB) (μ /ml)	4.79 $\pm$ 0.24	4.7 $\pm$ 0.48	NS
HDR (pre-CPB) (sec/ μ /ml)	68.7 $\pm$ 23.3	96.7 $\pm$ 40.8	0.062
Heparin resistant (% yes)	42.8	57.1	NS
Additional heparin pre-CPB (units)	4667 $\pm$ 11431	2312 $\pm$ 4615	NS
Heparin concentration (on-CPB) (μ /ml)	4.27 $\pm$ 1.38	4.09 $\pm$ 0.73	NS
Additional heparin on CPB (units)	10000 $\pm$ 8295	6375 $\pm$ 9113	NS
HDR (on-CPB) (sec/ μ /ml)	79.0 $\pm$ 23.8	107.4 $\pm$ 36.4	0.053
Lowest ACT (sec)	393.7 $\pm$ 84.6	464.9 $\pm$ 90.3	NS

ACT = activated clotting time; HDR = heparin dose response; HLD = heparin loading dose. All values mean $\pm$ SD, except for nominal data, which are shown as %.

Descriptive statistics were done to determine the characteristics of those patients with thrombocytosis. Knowledge of these characteristics may alert the perfusionists to the possibility of heparin resistance before the patient is heparinized. The patients with thrombocytosis were slightly smaller than the control patients, and as a result, had a smaller HLD (based on 300 μ /kg dose). The percentage of patients on heparin drips or NTG drips preoperatively was not different between the groups. The type of operation these patients underwent was significantly different. More patients in the thrombocytosis group were in the "other" category for procedure than were expected. The majority of these procedures were congenital repairs (i.e., atrial septal defects) performed on younger female patients. These patients may have been more likely to have been on oral contraceptives, which have been shown to cause hypercoagulability (4); however, this variable was not recorded in this study.

The patients with thrombocytosis had lower ACTs after the HLD, and subsequently, had lower HDR computed pre-CPB (68 sec/ μ /ml vs. 78 sec/ μ /ml). Because the ACTs after HLD were low, these patients required more heparin to initiate CPB. Once on CPB, and the effects of hemodilution and heparin in the prime were taken into account, the computed HDR in both groups increased and were no longer significantly different (98 sec/ μ /ml vs. 90 sec/ μ /ml); however, the thrombocytosis patients did require more heparin on CPB to maintain an ACT of 480 sec. Even with the additional heparin given, the lowest ACTs on CPB were lower in this group. This information confirms previous reports that increased platelet counts can cause heparin resistance.

As shown in Figure 4, not all of the patients with high platelet counts were heparin resistant, and not all of the patients with heparin resistance had high platelet counts. Therefore, other variables must be considered when determining the pre-

Platelet count vs. HDR

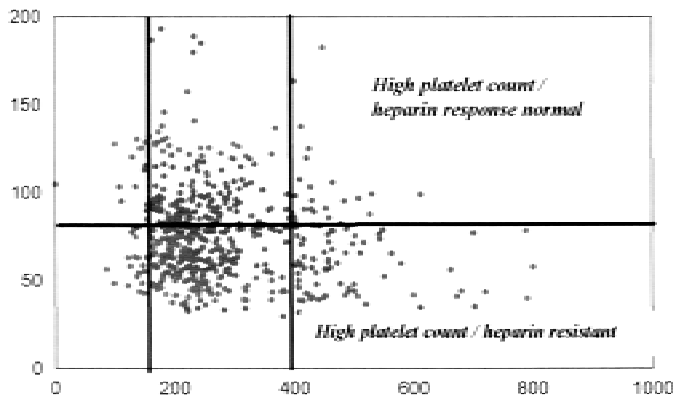


Figure 4: Scatterplot of the platelet count and the heparin dose response obtained pre-CPB. The grid lines mark the upper and lower limits of both platelet count (150 and 400 k/cumm) and the cut-off for heparin resistance (60 sec/ μ /ml).

dictors of heparin resistance. The variables studied in this project that have been listed as predictors in previous studies are age, heparin and NTG drips, and preoperative plasmapheresis.

Age was a significant univariate predictor of heparin resistance in this study. Increasing age increased the probability that a person would be heparin resistant. Previous studies have shown that both extremes of age are associated with heparin resistance: neonates and the elderly (5–7). This study only included adult patients, so no comments can be made on young ages and heparin resistance. The exact mechanism by which increasing age affects heparin sensitivity is not known.

Although the percentage of patients on heparin and/or NTG drips preoperatively was not different between the two groups, use of these drugs were significant univariate predictors of heparin resistance. This agrees with previous reports (8–14).

Use of plasmapheresis has been previously described as a method of preserving coagulation factors and platelets until after CPB (29–31). There has been one report of heparin resistance as a result of plasmapheresis (12). The patients in this study had normal preoperative platelet counts, and the authors hypothesize that removal of the PRP may have lysed platelets resulting in a release of platelet factor 3, a phospholipid that has been shown to accelerate several clotting times. There have been no reports of use of plasmapheresis to remove platelets in patients with thrombocytosis for the purpose of decreasing heparin resistance. In 22 patients who had plasmapheresis, six had high platelet counts. No significant differences were found in ACT after HLD, additional heparin necessary to go on CPB, additional heparin administered on CPB, and lowest ACT on CPB. Even with plasmapheresis, the HDR was still lower in the patients with high platelet counts; however, there was no statistical difference, probably because of the small sample size (low power). Even with the lower calculated HDR in this

group, the use of plasmapheresis did decrease the heparin required to initiate CPB as well as the heparin required on CPB to maintain a minimal ACT. The lowest ACT on CPB was still lower in the high platelet count group; however, the difference was not statistically significant. The HDR may not have been affected by removal of PRP for two reasons. First, if removal of PRP does result in activation of the platelets, the subsequent release of PF4 may overcome any benefits of removing the platelets. Second, it is possible that, in these patients, a significant amount of the platelets may not have been removed. It is possible that increasing the volume of PRP removed (and the subsequent number of platelets) could increase the patients HDR.

Although the incidence of thrombocytosis is relatively low, it is important for perfusionists to recognize that it can occur and may be a cause of heparin resistance during CPB. The treatment for this resistance is to increase the amount of heparin to overcome the antiheparin effects of the platelet factor 4. Use of plasmapheresis to remove some of the platelets before initiation of CPB may affect the incidence of heparin resistance in patients with high platelet counts; however, further studies with a larger sample size still must be done to determine conclusively whether using this technique to decrease the platelet count actually diminishes the frequency of heparin resistance and affects the clinical outcome of the patients.

Because this was a retrospective study, one limitation would be that all information came from the review of old pump records. Other information that may have been useful for this study include: amount of crystalloid volume anesthesia had given pre-CPB, total volume balance, resternotomy, and so forth, but were not routinely recorded on the pump records; therefore, were not available for this study. Despite these limitations, important conclusions can be drawn from these data.

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