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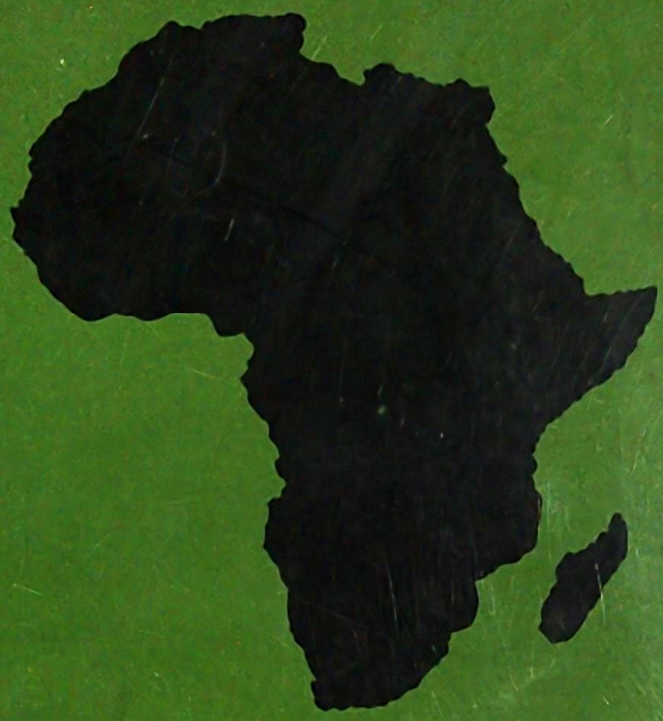

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Effects of minor surgery on some aspects of platelet function

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Summary

Blood from 33 patients undergoing elective surgery for non-malignant disorders was examined before operation and on the third post-operative day. The following parameters were measured:

(a) total platelet count
(b) platelet volume
(c) released adenine nucleotides (ATP and ADP).

Whole blood platelet count (WBPC) levels increased significantly after surgery and there was significant correlations between its pre- and post-operative values ($r = 0.616$, $p < 0.05$). The relationship between mean arithmetic volume after operation (MAVA) and the whole blood platelet count after operation (WBPC A) was negative and significant ($r = -0.425$, $p < 0.05$).

The results also revealed no significant differences between total and released nucleotides before and after surgery. The significant increase in whole blood platelet count post operatively may have been due to release of the splenic platelet stores. Platelets in the spleen are said to be old platelets hence the lack of a significant change in platelet volume may be due to sampling taking place three days after surgery which may not have corresponded to the time of the optimum production of young larger platelets. Also, the trauma of minor surgery may not have been strong enough to stimulate platelet production in the marrow.

In the light of the above findings further investigations may be conducted in which post-operative samples would be collected at varying intervals to determine whether post-operative changes in platelet volume occur at all and when.

Résumé

Le sang de 33 patients devant subir une opération facultative pour des affections non malignes a été examiné avant l'opération et le troisième jour après l'opération. On a mesuré les paramètres suivants:

(a) la numération totale des plaquettes
(b) le volume des plaquettes
(c) les nucléotides d'adénine libérés (ATP et ADP)

Les niveaux de la numération totale des plaquettes one augmenté significativement après l'opération et on a noté des corrélations significatives entre les valeurs pré-opératoires et post-opératoires ($r = 0,616$, $p < 0,05$). Le rapport entre le volume arithmétique moyen après l'opération et la numération totale des plaquettes après l'opération était négatif et significatif ($r = - 0,425$, $p < 0,05$).

Les résultats n'ont pas non plus mis en évidence des différences significatives entre les nucléotides totaux et ceux libérés, avant et après l'opération. L'augmentation significative dans la numération totale des plaquettes après l'opération peut avoir été due à la libération d'une accumulation de plaquettes spléniques. On considère que les plaquettes de la rate sont vieilles; il en découle que l'absence de changement significatif dans le volume des plaquettes peut être due au fait que l'échantillonnage a été effectué trois jours après l'opération, ce qui peut ne pas avoir correspondu à la période nécessaire pour une production optimale de jeunes plaquettes plus larges. De même, la blessure causée par une opération mineure peut ne pas avoir été assez importante pour stimuler la production de plaquettes dans la moelle.

A la lumière des constatations ci-dessues, on peut mener d'autres recherches au cours desquelles les échantillons après l'opération seraient reassemblés à des intervalles variés, afin de déterminer si vraiment des changements post-opératoires dans le volume des plaquettes interviennent et si oui, à quel moment.

Introduction

Platelets are of prime importance in haemostatis. During clotting, structural and physiological changes

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occur in the platelets and there also exists a relationship between platelet numbers, bleeding time and clotting. In the haemostatic interaction of platelets with blood vessels and plasmatic coagulation proteins, the development of platelet 'stickiness' in response to a variety of specific stimuli is a crucial step, allowing adhesion of platelets to surfaces, cohesion of platelets to each other, and the ultimate generation of a properly localised platelet-fibrin plug. It is also known that specific chemicals substances e.g. thromboxanes, calcium; adenine nucleotides secreted or liberated externally during platelet activation processes interact importantly both with platelets and with blood vessels. It has been observed that changes in platelet volume, count and adenine nucleotides also take place in patients with myocardinal infarction, disseminated intravascular coagulation and in neurosurgical patients with aneurysms as well as in severe post-surgical bleeding.[1,2,3].

To ascertain whether this is a general phenomenon in surgical patients this study was conducted to determine:

(a) Platelet count in patients before and after surgery;
(b) Platelet volume before and after surgery;
(c) Total and released platelet nucleotides before and after surgery; and
(d) The relationship among the above parameters. Such a study would help in identifying factors which may stimulate increased proliferation of platelets and add to our knowledge of influences exerted by certain platelet parameters on one another.

Materials and methods

Patients investigated were among those admitted to the surgical ward for elective surgery for non-malignant conditions such as hernia repair, cholecystectomy, appendicectomy and prostatectomy. Thirty-three patients aged between 21 and 89 years were randomly selected and they consisted of 14 females and 19 males.

Control blood samples were obtained from these patients before surgery. A careful history of any drug medication within a week prior to the sampling was taken from each patient particularly drugs that could affect platelet aggregation such as aspirin and other non-steroidal anti-inflammatory drugs. 30ml of blood were obtained by clean venepuncture preferably without venous occlusion from the anterior cubital vein. Disposable plastic syringes and siliconised 19G or 21G needles were used.

Estimates of aggregation, nucleotide, platelet volume and whole blood platelet count were conducted before operation and on the third post-operative day.

Platelet aggregation

This was measured using an Accutech Aggregometer attached to a Linseis 04802 flat bed chart recorder. The instrument was first calliberated by placing one ml each of the platelet rich plasma and corresponding platelet poor plasma were placed in separate cuvettes and a stir bar placed in each. Each was then used in turn to present the minimum and maximum levels of light transmission for platelet rich plasma and platelet poor plasma respectively. For the actual aggregation test, 1ml of platelet rich plasma was pre-warmed in a cuvette to 37°C, a stir bar was placed in it and 20 µl of collagen was then added and the aggregation was recorded. This procedure was repeated using 5 µl of collagen. All aggregation experiments were performed at 37°C and completed within three hours of blood collection.

Platelet volume and count

Nine ml of blood, anticoagulated with 1ml of dipotassium EDTA, was spun at 22°C in an MSE chilspin centrifuge at 1000 rpm for 10 minutes to obtain platelet rich plasma. 20 µl of the platelet-rich plasma was taken midway between the meniscus level and red cell layer. This was diluted in 20 ml of isoton 11 solution in coulter pots. The resulting suspension was electronically counted to obtain a platelet count for the platelet rich plasma.

The platelet-rich plasma was adjusted with isoton 11 solution to give a platelet count of between 280 to 320 x 10^9/l for the platelet volume 10^9/l determination. The electronic counting was completed within 10 minutes of the dilution and between two and four hours after blood collection. Platelet volume was determined using a coulter counter model ZF in conjunction with a C1000 channelyzer linked directly to a microcomputer. Results were recorded by a teleprinter. Whole blood platelet count was performed using a Technicon automated platelet counter.

Platelet Aggregation

One ml of platelet rich plasma was taken in each

case. Twenty µl of collagen was then added to the platelet rich plasma for released nucleotides determination. The preparation was left for four minutes after which it was spun in a micro-angle centrifuge at 6000 r.p.m. for five minutes and then carefully decanted into another glass test-tube. Platelet rich plasma samples for both total and release nucleotides were henceforth treated similarly.

One hundred µl EDTA was added to each preparation to stop the release reaction. One hundred µl of triton was also added to prevent, together with EDTA, further conversion of nucleotides by nucleotide converting enzymes and then left to stand for two minutes at room temperature after which 800 µl of ethanol was added to precipitate proteins.

The bioluminescent assay technique was utilized to determine total adenine nucleotides contents as well as the amount of each nucleotide released into plasma following platelet stimulation. Use was made of luciferin/luciferase reagents which give a constant light output with time. In combination with nucleotides the light output which can be detected with an ATP monitoring reagent (L.K.B. - Wallac) is proportional to ATP concentration over the range 10^{-11} to 10^{-6} M. The basis of luciferin-based techniques depends on the following reaction:-

ATP + luciferin + O_2 ---------- Oxyluciferin + AMP
+ PPi
+ CO
+ Light

ATP and ADP content of platelets was measured directly by a combined ATP/ADP bioluminescent assay and the following basic reaction resulting from the addition of enzyme to the nucleotide extracts:

Nucleotide extract + active pyruvate kinase = ATP
(ATP + ADP + Phosphoenolpyruvate = ATP +
ATP + Pyruvate

ADP is converted to ATP by the enzyme pyruvate kinase using phosphoenol pyruvate. Estimations of nucleotide content were then made according to the formulae suggested by Summerfield *et. al.* [4]

Statistical Analysis

T-tests and linear correlation coefficients (r) were estimated according to procedures outlined by Swinscow[2]. The significance of a difference in a variable between its mean value before (B) and after (A) surgery was determined using the appropriate t-test. Linear correlation coefficients were estimated to determine the relationship between the values of parameters before and after surgery as well as among the various parameters.

The paramaters of interest included:

(a) whole blood platelet count (WBPC)
(b) Platelet volume. Comparisons involving platelet volume were based on mean long values (MLV) and mean arithmetic values (MAV). Log transformation of platelet volume was utilized because transformed platelet volume data followed a normal distribution,[6].
(c) Total (T) and release (R) ATP and ADP. Some comparisons were also made between WBPC and platelet mass (PM).

Results

Whole blood platelet count (WBPC) increased significantly after surgery with a value of 313.24 compared to 274.48 before surgery (Table 1).

However, there were no significant differences between pre- and post-surgery values of platelet volume, platelet mass and platelet adenine nucleotides (Table 1).

Table 1: Values of platelet parameters before and after surgery

Parameter	Mean Value	
	Before	After
WBPC	274.48 ± 18.80	313.24 ± 21.41*
MLV	0.88 ± 0.01	0.87 ± 0.01 NS
MAV	8.79 ± 0.23	8.66 ± 0.17 NS
PM	2373.10 ± 150.59	2662.60 ± 157.03 NS
TATP	26.29 ± 1.52	26.59 ± 1.33 NS
TADP	17.32 ± 1.20	18.53 ± 1.02 NS
RADP	8.49 ± 0.78	8.54 ± 0.62 NS
RADP	11.14 ± 1.15	10.48 ± 0.66 NS

*Significant difference between values before and after surgery ($P < 0.05$).
NS = No significant difference.

Linear correlation coefficients among platelet parameters are shown in Table 2. There were significant correlations between pre-surgery and post-surgery values of parameters such as WBPC MLV and MAV. The high correlation between mean

log volume (MLV) and MAV (mean arithmetic volume) values both before and after surgery indicate the close association between original and transformed data which may be expected.

Table 2: Co-efficients of linear correlation (r) among platelet paramaters

		TATPB	TATPA	TADPB	TADPA	RATPB	RATPA	RADPB	RADPA	WBPCB	WBPCA	MLVB	MLVA	MAVB
	TATP B	—												
	TATP A	0.16	—											
Nucleo-	TADP B	0.84**	0.1	—										
tides	TADP A	0.20	0.60**	0.28	—									
	RATP B	0.71	0.18	-0.64**	0.21	—								
	RATP A	-0.20	0.37*	-0.27	0.12	-0.25	—							
	RADP B	0.76**	0.11	0.80*	0.13	0.85*	0.35	—						
	RADP A	0.02	0.66*	0.08	0.52*	-0.09	0.66**	-0.06	—					
Platelet	WBPC B	-0.27	0.02	-0.35**	-0.05	-0.19	0.11	-0.28	0.11	—				
count	WBPC A	-0.22	-0.03	0.38*	0 02	-0.08	0.04	-0.20	-0.07	0.62*	—			
	MLV B	0.11	0.40*	0.08	0.19	0.07	0.06	0.12	0.26	-0.27	-0.31	—		
Platelet	MLV A	0.10	0.22	0.22	0.10	-0.09	0.11	-0.04	0.21	-0.39*	-0.50**	0.59**	—	
Volume	MAV B	0.07	0.38*	0.02	0.19	0.06	0.26	0.09	0.21	-0.29	-0.27	0.99**	0.99*	—
	MAV A	0.12	0.25	0.02	0.13	-0.07	0.13	-0.03	0.22	-0.34	-0.43*	0.59*	0.99	0.56*

* Significant (P < 0.05) ** Significant (P < 0.01).

However, of greater interest are the significant relationships between different parameters. Such relationships were recorded between WBPCB and TADPB (r = -0.331*) between WBPCA and TADP B(r = -0.38*); TATPA with MLVB (r = 0.40*); TATPA and MAVB (r = 0.38*); MLVA with WBPCB (r = -0.43*). It is noteworthy that the relationships between platelet volume and whole blood platelet count both before and after surgery exhibited negative trends, even though not all were significant.

Platelet mass values before and after surgery were significantly related (r = 0.55**) as were WBPCB and PMA (r = 0.56**); WBPC before and after surgery were highly correlated with platelet mass before (r = 0.92**) and after surgery (r = 0.95**), respectively (Table 3).

Table 3: Coefficients of linear correlation between whole blood platelet count and platelet mass before and after surgery

	WBPC B	WBPC A	PMB
WBPCB	—		
WBPCA	0.62**	—	
PMB	0.92**	0.52**	—
PMA	0.56**	0.95*	0.55*

* Significant (P < 0.05)
** Significant (P < 0.01)

Discussion and conclusions

The significant inverse relationship between platelet number and platelet volume recorded after surgery in this study has also been reported by O'Brien and Jamieson[7], Paulus[6] and Paulus *et. al.* [8]. The same relationship has also been recorded from animal studies[9].

McDonald *et. al.* [10] claimed that under certain

clinical conditions, young platelets are larger than older ones. In this study, there was no increase in platelet volume after surgery even though platelet count increased. It may be that the platelets that brought about an increase in number after surgery were not large enough to cause a significant increase in volume. Also, by taking blood three days post-operatively, the period when the marrow produces the large young platelets might have been missed. Sequential sampling at frequent intervals might help indicate the time of optimum production of young larger platelets. Garg *et. al.* [11] who measured platelet volume two hours after surgery recorded increased in 4 out of 22 patients. Yamazaki *et. al.* [12] also suggested that the large younger platelets are consumed early in thrombosis and haemostatic plug formation after an operation.

Another explanation for the non-significant change in platelet volume is that the splenic pool of platelets may have released its store because of the stress of surgery; in this situation, platelet volume would not be affected since platelets stored in the spleen are relatively old and therefore small-sized.

It is not as yet established whether or not platelet volume changes significantly following surgery. Platelet volume analysis could be exploited clinically as a diagnostic prognostic or therapeutic index since the paramater has been shown to be abnormal in patients with some clinical disorders e.g. myeloproliferative diseases Cottier *et. al.*, unpublished data[1].

Von Behrens[9], suggested that the number of circulating platelets is irrelevant in any clinical state but that what is important is the total mass of circulating platelets which regulates platelet production. In the present study, there was no significant increase in platelet mass following surgery. Although this result suggests that the marrow had not been sufficiently stimulated to produce large numbers of new platelets, it may also be related to the time of production and consumption of young larger platelets as discussed above.

The lack of a significant change in the amount of adenine nucleotides after surgery in this study may be related to the absence of a significant change in platelet volume. Large platelets have an increase store of adenine nucleotides[13] but these large platelets were apparently not produced or could not be detected owing to the timing of platelet volume determination.

Platelet volume before surgery can be used as a predictor or post-operative ATP because of their positive correlation. Similarly, ADP content before surgery can predict WBPC after surgery owing to their significant negative relationship.

While the results in this study may not be conclusive, they are sufficiently interesting to form the basis of further work particularly with respect to methodology in clearly establishing whether post-operative changes in platelet volume occur at all and when.

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