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Serial Determinations of PF4 and βTG: Comparisons Between Multiple Venipunctures vs a Catheter Infusion System

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Summary

Platelet factor 4 (PF4) and beta thromboglobulin (βTG) are platelet-specific proteins which are released upon platelet aggregation and which can be accurately measured by radioimmunoassay. We devised a catheter-infusion system that enables serial determinations of these proteins.

In 20 subjects (10 healthy volunteers and 10 patients with stable coronary artery disease), we compared samples collected by individual venipunctures with those simultaneously obtained by means of a simple catheter-infusion system. At least 5 samples were obtained over a period of time which was as long as 60 min, and at least 30 min. Subjects with stable coronary artery disease were selected so that they would be expected to have stable and normal PF4 and βTG levels. Thus, elevations of either PF4 or βTG would represent artifacts secondary to sampling technique.

Analysis of the results demonstrated that the catheter-infusion system was equivalent to individual venipunctures for determination of PF4 and βTG. 16.8% of samples obtained via the catheter and 17.2% of those obtained by individual venipunctures were spuriously elevated.

A second series of studies were performed to refine the technique further by examining the impact of infusion rate and the addition of citrate phosphate dextrose (CPD) to the infusate. Ten additional subjects had catheter systems utilized in both arms simultaneously. The addition of CPD resulted in significantly less abnormal values at slower infusion rates (1 and 2.5 cc/min). At 5 cc/min D5/w or saline alone are suitable.

These investigations confirm that this simple catheter system is equivalent to individual venipunctures for determination of PF4 and βTG while avoiding patient discomfort. Also noted was the fact that a high percentage of determinations could be spuriously elevated by either technique under clinical demands. Thus, multiple determinations from the same subject are necessary to assure reproducibility.

Introduction

For the past decade there has been heightened interest in the potencial roles played by platelets in the pathophysiology of ischemic coronary disease. There is obvious need for an accurate and reproducible test of platelet activation. Platelet factor 4 (PF4) and beta thromboglobulin (βTG) are platelet-specific proteins that are released from α granules during platelet activation. Levels of these proteins can be accurately measured by radioimmunoassay. Prior work has emphasized the critical importance of technical and methologic factors in measuring these factors (1–3). Previously we devised a catheter-infusion system that enabled serial sampling of blood for determination of PF4 and βTG (2). We have previously utilized this system in an investigation of exercise-induced myocardial ischemia. By means of the serial sampling afforded by this technique we demonstrated that increases in PF4 and βTG that occurred were secondary to technical factors (2). In the prior study we had confirmed the accuracy of this technique compared to individual venipunctures in a limited number of patients.

The goals of the present study were several: in a larger sample of patients and normal volunteers, confirm the accuracy of the catheter-infusion system vs individual venipunctures, establish the optimal rate of infusion and type of infusate used and elucidate whether the addition of the anticoagulant CPD enhanced the technique.

Materials and Methods

Experimental Design

The investigation consisted of two phases. In the first portion 20 subjects, 10 patients with coronary artery disease and 10 normal volunteers, had blood collected initially and serially for at least 30, and as long as 60 min. Sampling was performed by means of individual venipunctures in one arm simultaneously while blood was collected from the opposite arm through the catheter system. Randomly, half of the subjects had CPD added to the infusate, which was normal saline at a rate of 5 cc/min. CPD (citrate phosphate dextrose), while an anticoagulant does not directly block platelet activation; however, it could prevent fibrin formation and platelet activation on the tip of the catheter.

The second phase of our study attempted to refine the catheter-infusate system into a technique that would be potentially better tolerated by cardiac patients than a rapid infusion of saline. Ten subjects had catheter-infusate systems employed in both arms and samples were obtained over a 30 min period. Five percent dextrose in water was substituted for normal saline in all infusions; however, into one arm CPD was added to D5/w, while the opposite arm's system utilized D5/w alone. Three different rates of infusion were utilized, 5, 2.5 and 1 cc/min. As during the first phase, simultaneous samples were obtained for PF4 and βTG.

Subjects

Ten males, aged 60–71 (mean 64.9) with known coronary artery disease participated in the study. Six had undergone cardiac catheterization (5 had three vessel disease; 1 had two vessel disease), 5 had a prior myocardial infarction, 5 had prior coronary bypass surgery and all 10 had angina on exertion. Medications included assorted antianginals, in addition 4 patients were taking aspirin daily.

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The 10 healthy volunteers ranged in age from 25 to 65 (mean = 40.9 years). Eight were male, and 2 female. Three were current smokers, while 1 subject had medication-controlled hypertension. This subject subsequently was eliminated from data analysis because of all determinations of PF4 and βTG being in the abnormal range. Three subjects were taking ASA, while 2 additional subjects were receiving nonsteroid anti-inflammatory agents.

The subjects for the second phase of the trial had a mean age of 40.8 years (31–62). Two had catheterization-documented coronary artery disease and one additional subject had hypertension. ASA and nonsteroidals had been taken within the prior 10 days by 1 subject each.

The protocol was approved by the Human Studies Committee and each subject gave informed consent.

Catheter-Infusate System

Local anesthesia was obtained with 1% lidocaine. A Teflon-coated No. 14G catheter (Abbocath-T), 2.5 inches in length and 0.15 cm in internal diameter was inserted into the medial basilic vein. To ensure sampling ease this catheter was connected via a 6-inch extension set (Abbott extension set Int-SL) and large-bore extension tubing to a prepared bottle of infusate.

In the first phase of the study this infusate was normal saline in half of the subjects and a mixture of 1 part CPD to 9 parts saline for the remainder. Choice of infusate was by random allocation but was administered in a non-blinded fashion. This infusate was administered at a rate of 5 cc/min by means of an IVAC pump. During the study approximately 150 cc of saline was administered.

The second phase of the trial further explored the impact of the addition of CPD to the infusate, as well as rate of infusion. The infusate was D5/w for all subjects; however, sampling from both arms was accomplished by means of the catheter-infusate system. The infusate into one arm was D5/w alone, while the other arm received a mixture of 1 part CPD to 9 parts saline. The rate of infusion for 2 subjects was 5 cc/min, for 5 subjects-2.5 cc/min, and the remaining 3 subjects received an infusion rate of 1 cc/min.

Specimen Collection

Samples were drawn at baseline and at 5, 10, 15 and 30 min. Two subjects had additional samples obtained at 45 and 60 min. Since we had previously determined that use of a stopcock greatly increased the likelihood of spurious elevations of PF4 and βTG, no stopcocks were utilized. Therefore the infusion was temporarily discontinued and blood obtained utilizing a two syringe technique. The first 3 cc of blood was drawn and discarded, and 4.5 ml of blood was drawn into a polypropylene syringe, which had been precooled in ice and contained 0.5 ml of anticoagulant in a final concentration of 12% acid citrate dextrose (NIH formula A), 1 mM ethylenediamine tetraacetic acid (EDTA) (Sigma, St. Louis, MO), 10 μM adenosine (Sigma, St. Louis, MO) and prostaglandin E_1 1 μ/ml (Sigma, St. Louis, MO).

Simultaneously, with each of the above samples, the opposite arm was entered with a clean venipuncture without use of tourniquet by a 21 gauge butterfly needle. An identical 2 syringe sampling technique was utilized. The initial blood drawn during the first and last samplings was sent for platelet count.

The syringes were immediately placed in melting ice for no longer than 30 min, and the blood was then transferred to a 12 × 75 mm plastic tube and centrifuged at 48,000 × g for 20 min at 4 °C. By means of a plastic tip 1 cm below the top layer of plasma, 0.5 ml of platelet-poor plasma was carefully aspirated and then stored at 20 °C for up to 3 weeks before assay.

Platelet Factor 4 and β-Thromboglobulin Radioimmunoassay (RIA) Kit Assay

The levels of PF4 and βTG, expressed in nanograms/ml (ng/ml), were determined via two commercial radioimmunoassays: PF4 (Abbott Laboratories, North Chicago, IL) and βTG (Radiochemical Center, Amersham, Buckinghamshire, England). The details of the methods used to determine PF4 and βTG levels have been described elsewhere (4, 5).

Platelet Concentration

The platelet concentration was determined with the Coulter Counter Model ZF (Coulter, Hialeah, FL) (6). The platelet concentration was expressed in $\times 10^9$/liter.

Statistical Analysis

For purposes of statistical analysis the data for PF4 and βTG were categorized into normal vs. abnormal scores in accordance with the following criteria:

Variable	*Normal Range*	*Abnormal Range*
PF4	0–10 ng/ml	>10 ng/ml
βTG	0–35 ng/ml	>35 ng/ml

The aim of data analysis was to examine whether abnormal scores occurred with greater frequency when the catheter method, rather than the venipuncture method of sampling was used. Since all subjects were stable, extreme readings would represent random events or errors in measurement, rather than true states associated with the subjects. Thus, although each subject's data yielded a number of observations, it was assumed that, within each individual, observations could be regarded as independent. Under this assumption the data were analyzed by means of a chi-squared test to examine whether the probability of abnormal measurement differed significantly as a function of measurement method.

Table 1 PF4 determinations

Subject	Catheter Sample Number					Venipuncture Sample Number				
	1	2	3	4	5	1	2	3	4	5
With CPD										
1	N	N	N	N	N	A	A	N	N	N
2	A	N	N	N	N	N	N	N	A	N
3	N	N	N	N	N	N	N	N	N	N
4	N	–	N	N	A	N	–	N	A	N
5	N	A	N	N	N	N	N	N	N	N
6	N	N	N	N	N	N	N	N	N	N
7	A	A	A	N	N	A	A	N	A	N
8	N	N	N	N	N	N	N	N	N	N
9	N	N	N	N	N	A	N	N	N	N
Without CPD										
1	A	N	N	N	N	N	N	N	N	N
2	A	A	N	N	N	A	N	A	N	N
3	A	N	N	N	N	N	N	N	N	N
4	N	A	A	A	N	N	A	–	A	N
5	N	N	N	N	N	N	–	N	N	–
6	N	N	N	N	N	N	N	A	N	N
7	N	A	N	N	A	N	N	A	A	N
8	N	N	N	N	N	N	N	N	N	N
9	N	N	A	N	N	N	N	N	N	A
10	N	N	N	N	N	N	N	N	N	N

A = Out of range; N = Within normal limits

Results

The overall goal of this investigation was to optimize the techniques for serial determinations of the platelet-specific proteins PF4 and βTG. Subjects were selected therefore, who were either healthy volunteers or patients with stable coronary artery disease. Thus, elevation of either PF4 or βTG would represent artifacts secondary to the sampling technique. One subject, a volunteer, healthy except for medication-controlled hypertension, had each and every determination of PF4 and βTG out of range. Since this would dilute the results, his data points were eliminated from analysis.

Comparison of Catheter-Infusate System vs. Individual Venipunctures

A total of 188 determinations of platelet factor 4 were obtained from 19 subjects (Table 1). Sixteen of the 95 samples obtained via the catheter system were abnormal (16.8%), while 16 of the 93 determinations from individual venipunctures were spuriously elevated (17.2%) (Table 2). There did not appear to be any relationship to time with respect to abnormal determinations, i. e. the incidence of elevated levels did not increase in the latter samples.

There was a similar lack of significant difference between the two techniques for levels of βTG. Twenty-three percent of the catheter samples were abnormal compared to 17.6% of those from individual venipunctures (Table 2). One patient had consistently elevated levels. This patient had chronic renal failure which may affect the clearance of βTG and increase the likelihood of elevated levels. With elimination of his data the percentage of abnormal determinations were 19% for catheter and 13% for venipunctures.

These original subjects had randomly been assigned to receive CPD added to the infusate or saline alone. There was no difference in the likelihood of abnormal determinations with or without the addition of CPD when the infusate was administered at a 5 cc/min rate (Table 3).

Comparison of D5/w alone vs. D5/w-CPD Mixture at Varying Infusion Rates

The initial portion of the study documented that the catheter-infusate system utilizing normal saline at 5 cc/min performed at least as well as individual venipunctures. The second phase further refined the technique by examining the impact of the addition of CPD to Dextrose and water, and the importance of infusion rate. Each of the 10 subjects had the catheter-infusion system utilized in both arms simultaneously; one system employed D5/w alone, the other D5/w with CPD added. Two subjects received the infusate at the original 5 cc/min, 5 at 2.5 cc/min, and 3 at 1 cc/min. When all 10 subjects are considered together, the addition of CPD provided a highly significant reduction in the occurrence of spurious elevations (Table 4). When the results are scrutinized for each of the three infusion rates, it is apparent that the benefit of the addition of CPD was noted only at the slower rates, 1 and 2.5 cc/min (Table 5). Although the number of determinations is small, it appears that D5/w at a rate of 5 cc/min is adequate to ensure a suitable method for serial determinations (Table 5).

Table 2 Catheter vs. venipuncture: All determinations

	Catheter	Venipuncture	Total
PF4 determinations			
Number normal	79	77	
Number abnormal	16	16	
Total number	95	93	188
% Abnormal	16.8%	17.2%	
Chi-square p = 0.95			
βTG Determinations			
Number normal	73	75	
Number abnormal	22	16	
Total number	95	91	186
% Abnormal	23% (19%*)	17.6% (13%*)	
Chi-square p = 0.35			

* With elimination of subject with mild chronic renal failure

Table 3 Catheter vs. venipuncture: Saline vs. CPD/saline

	Catheter	Venipuncture	Total
PF4 determinations with CPD			
Number normal	38	37	
Number abnormal	6	8	
Total number	44	45	89
% Abnormal	13.6%	17.8%	
Chi-square p = 0.59			
PF4 determinations without CPD			
Number normal	41	40	
Number abnormal	10	8	
Total number	51	48	99
% Abnormal	19.6%	16.7%	
Chi-square p = 0.70			
βTG determinations with CPD			
Number normal	33	36	
Number abnormal	11	7	
Total number	44	43	87
% Abnormal	25%	16%	
Chi-square p = 0.32			
βTG determinations without CPD			
Number normal	40	39	
Number abnormal	11	9	
Total number	51	48	99
% Abnormal	21.6%	18.8%	
Chi-square p = 0.73			

Discussion

Platelets continue to play an increasingly important role as factors involved in the development of atherosclerosis and its complications. They appear to be important in the development of atherosclerotic lesions themselves, formation of thrombus upon already present lesions and the release of vasoactive substances that further dynamically affect coronary flow. In vivo measurement of platelet activation has long been considered as a marker of such dynamic processes. Assays for the platelet specific proteins platelet factor 4 (PF4) and beta thromboglobulin (βTG) have been documented to indicate ongoing platelet activation.

Obviously, for many situations such as ongoing myocardial ischemia or evolving myocardial infarction, the time course of a series of such measurements of platelet aggregation would be of greater interest than isolated determinations. We undertook to develop a methodology that would permit multiple and serial sampling for these factors, avoiding individual venipunctures

Table 4 Catheter system: CPD/D^5/w vs. D^5/w

	With CPD	Without CPD	Total
PF4 determinations; N = 10			
Number normal	49	30	
Number abnormal	1	15	
Total number	50	45	95
% Abnormal	2%	33%	
Chi-square p = 0.0001			
βTG determinations; N = 10			
Number normal	44	21	
Number abnormal	6	24	
Total number	50	45	95
% Abnormal	12%	53%	
Chi-square p = 0.0001			

Table 5 Catheter system: Varied rate of infusate

	With CPD	Without CPD	Total
PF4; N = 3; 1 cc/min			
Number normal	15	10	
Number abnormal	0	5	
Total number	15	15	30
% Abnormal	0	33%	
Chi-square p = 0.014*			
βTG; N = 3; 1 cc/min			
Number normal	11	6	
Number abnormal	4	9	
Total number	15	15	30
% Abnormal	26.7%	60%	
Chi-square p = 0.06			
PF4; N = 5; 2.5 cc/min			
Number normal	24	11	
Number abnormal	1	9	
Total number	25	20	45
% Abnormal	4%	45%	
Chi-square p = 0.001*			
βTG; N = 5; 2.5 cc/min			
Number normal	23	7	
Number abnormal	2	13	
Total number	25	20	45
% Abnormal	8%	65%	
Chi-square p = 0.001			
PF4; N = 2; 5 cc/min			
Number normal	10	9	
Number abnormal	0	1	
Total number	10	10	20
% Abnormal	0	10%	
Chi-square p = 0.30*			
βTG; N = 2; 5 cc/min			
Number normal	10	8	
Number abnormal	0	2	
Total number	10	10	20
% Abnormal	0	20%	
Chi-square p = 0.14*			

* Note: Over 20% of the cells have expected counts less than 5. The table is so sparse that chi-square may not be a valid test.

whose number of samplings would be limited by patient comfort and venous access.

Catheter-Infusate System

The results from our comparison of the catheter system to individual venipunctures confirms our prior conclusion that this method permits multiple and serial determinations for these platelet specific proteins. Either saline or D5/w are suitable infusates and when infused at a rate of 5 cc/min do not appear to benefit significantly from the addition of the anticoagulant CPD. However, at the slower rates of 1 and 2.5 cc/min the addition of CPD appeared to prevent artifactual elevations of PF4 and βTG, presumably by preventing clotting on the tip of the catheter.

Reproducibility of PF4 and βTG Determinations

Prior work from our laboratory (2) as well as from other labs (1, 3) have emphasized the critical role that technical considerations may play in measurements of PF4 and βTG. Thus, technical factors were responsible for elevations in myocardial infarction patients (3) as well as patients developing exercise-induced myocardial ischemia (2). Technical difficulties must be questioned whenever the results of such studies reach conclusions that in part are at variance with the expected. Thus, as an example, while Levine and coworkers noted increases in PF4 and βTG in medical residents in association with the emotional stress of lecturing, the conclusion might be questioned since the subjects also had abnormally elevated values during the second control period, 5 to 21 days after stress (7).

Many laboratories, including ours, are sensitive to the need for meticulous technique. Indeed, our current values of normal for PF4 are not 0–10, but rather a narrower range of 0–5 ng/ml. However, we feel that the present study demonstrates that while such low values for PF4 and βTG should be expected for individual determinations in the controlled laboratory situation, in *clinical situations,* as many as 15% of determinations may be expected to be abnormal.

Thus, while a single value may be sufficient under ideal laboratory conditions, in clinical situations, and especially if time constraints are adding additional pressure, there is need for either a larger series of patients or serial determinations from the same patient, before conclusions reached can be valid.

The above caveats concerning technique hold true for either individual venipunctures or the catheter-infusion system. The latter technique obviates the potential need for tourniquets and permits multiple sampling.

References

1 Files J C, Malpass T W, Yee E K, Ritchie J L, Harker L A. Studies of human platelet α-granule release in vivo. Blood 1981; 58: 607–18.

2 Strauss W E, Cella G, Parisi A F, Sasahara A A. Serial studies of platelet factor 4 and beta thromboglobulin during exercise in patients with coronary artery disease. Am Heart J 1985; 110: 293–9.

3 Jaffe A S, Lee R G, Perez J E, Geltman E M, Wilner G D, Sobel B E. Lack of elevation of platelet factor IV in plasma from patients with myocardial infarction. J Am Coll Cardiol 1984; 4: 653–9.

4 Handin R I, McDonough M, Lesch M. Elevation of platelet factor four in acute myocardial infarction: Measurement by radioimmunoassay. J Lab Clin Med 1978; 91: 340–9.

5 Bolton A E, Ludlam C A, Moore S, Pepper D S, Cash J D. Three approaches to the radioimmunoassay for human β-thromboglobulin. Br J Haematol 1976; 33: 233–8.

6 Cella G, Colby S I, Taylor A D, McCracken L, Parisi A F, Sasahara A A. Platelet factor 4 (PF4) and heparin released platelet factor 4 (HR-PF4) in patients with cardiovascular disorders. Thromb Res 1983; 29: 499–509.

7 Levine S P, Towell B L, Suarez A M, Knieriem L K, Harris M M, George J N. Platelet activation and secretion associated with emotional stress. Circulation 1985; 71: 1129–34.

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