

ORIGINAL ARTICLE

INCREASED RISK OF THROMBOEMBOLISM AS A RESULT OF HIGH THROMBIN PRODUCTION IS ASSOCIATED WITH SHORT ACTIVATED PARTIAL THROMBOPLASTIN TIME IN CANCER PATIENTS ON AND AFTER CHEMOTHERAPY: A PROSPECTIVE STUDY IN A TERTIARY CARE HOSPITAL

Indranil Dhar¹, Debkumar Ray², Debashis Roy Burman³, Shibashish Bhattacharyya⁴

HOW TO CITE THIS ARTICLE:

Indranil Dhar, Debkumar Ray, Debashis Roy Burman, Shibashish Bhattacharyya. "Increased Risk of Thromboembolism as a Result of High Thrombin Production is Associated with Short Activated Partial Thromboplastin Time in Cancer Patients on and After Chemotherapy: A Prospective Study in a Tertiary Care Hospital". *Journal of Evolution of Medical and Dental Sciences* 2014; Vol. 3, Issue 25, June 23; Page: 6932-6937, DOI: 10.14260/jemds/2014/2842

ABSTRACT: To investigate whether cancer patients during and following chemotherapy with short activated partial thromboplastin times (aPTTs) have increased thrombin generation and are at increased risk for thromboembolism, this prospective study was designed. Routine coagulation specimens of such patients were screened for the presence of short or normal aPTT for 5-month period, and, accordingly, 250 specimens were collected. Prothrombin fragment F1+2 (F1+2) was measured to evaluate thrombin activation, and a second aPTT was performed with a different reagent. Clinical history were obtained from medical records after conclusion of sample collection. 6 to 12 months later, patients were questioned on thromboembolic events during the previous 18 months by questionnaire. F1+2 and the incidence of venous thromboses were elevated significantly in the short aPTT group. Patients with acute bleeding had short aPTTs, but 36% of these also had thromboembolic events during the 18 months proximal to blood collection. These findings were confirmed with the second aPTT reagent. Patients with short aPTTs have increased thrombin generation and are at increased risk for thromboembolism, mainly venous thromboses, despite the fact that a short aPTT can occur in the acute setting of bleeding.

KEYWORDS: Thromboembolism, activated partial thromboplastin time, chemotherapy.

INTRODUCTION: The prothrombin time (PT) and the activated partial thromboplastin time (aPTT) are routine coagulation tests used to assess the coagulation system in a clinical setting. Numerous factor deficiencies are known to prolong the respective screening test result and can be related to bleeding;⁽¹⁾ however, prolongation of the aPTT may be related to a hypercoagulable state if a lupus anticoagulant is present.⁽²⁾ A clinically relevant decrease in the activity of clotting factors usually related to the prolongation of one of the respective routine screening test results. By analogy, shortened clotting times could, therefore, be the expression of a hypercoagulable state. Earlier studies have found some evidence that short aPTTs are related to a higher incidence of thromboembolic disorders.^(3, 4) These findings have been disputed, and there is some suggestion in the literature that short aPTTs occur during bleeding episodes.^(5, 6)

Nowadays, molecular markers of activation processes in the coagulation and fibrinolytic system have become widely available for use in the specialized clinical laboratory. Prothrombin fragment F1+2 is cleaved from prothrombin on activation by factor Xa in amounts equimolar to the thrombin generated.⁽⁷⁾ Consequently, F1+2 levels have been shown to reliably measure the amount of prothrombin activation and, thus, thrombin generation.⁽⁸⁾

The purposes of the present study were to evaluate the hypothesis that in cancer patients

ORIGINAL ARTICLE

during and after chemotherapy, short aPTTs (ie, aPTTs below the lower limit of the reference range) are related to an increase in F1+2 levels and to relate these results to the findings of a patient survey on thromboembolic events. If short aPTTs were indeed related to a hypercoagulable state, this finding could be helpful for detecting subpopulations of patients at higher risk for thromboembolic disorders.^(9,10)

MATERIALS AND METHODS: During a 5-month period, specimens of inpatient and outpatient cancer patients undergoing chemotherapy or in post chemotherapy follow-up specimens were sent to the coagulation laboratory at School of Tropical Medicine, Kolkata, for routine coagulation studies and were screened for the presence of a normal or a short aPTT. Blood collection for routine blood tests was performed by phlebotomists or nurses. Blood for testing was collected into a 0.129-mol/L concentration of buffered sodium citrate (9 parts blood/1 part citrate, vol/vol) using the 2 tubes (Vacutainer, Becton Dickinson, Rutherford, NJ) technique. Platelet- poor plasma was prepared by centrifuging at 1600g and 22°C for 10 minutes. According to standard laboratory policy, a new sample was requested if the tubes were not filled adequately or if moderate to severe hemolysis or any sign of clot formation was present.

As a prospective study, we defined short aPTTs as a specimen exhibiting an aPTT of less than 24.8 seconds. A normal aPTT, for study purposes, was defined as 30.7 ± 2 seconds. The normal reference range for the aPTT at School of Tropical Medicine, Kolkata, 24.8 to 36.6 seconds, was established by testing plasma from 50 healthy volunteers. Short aPTT values were confirmed by repeated testing on the same specimen. Samples were screened and, after meeting the aforementioned criteria, were selected for inclusion into the study. We planned to collect equal numbers of specimens with normal and short aPTTs to a total of 250 samples.

Determination of the routine aPTT was done with the automated aPTT reagent from OrganonTeknika (Durham, NC) on an ACL 3000 Plus (Instrumentation Laboratories, Lexington, MA). The remaining plasma was used for Batch analyses for F1+2 (Enzygnost F1+2 micro, Behringwerke, Marburg, Germany) were performed later on these samples. All tests were performed according to the manufacturer's recommendations.

To recognize a possible bias related to the aPTT reagent used, the results obtained with the routine aPTT reagent were compared with those obtained with a different aPTT reagent (Pathromtin SL, Behringwerke, Marburg, Germany), also run on an ACL 3000 Plus. A reference range for this second aPTT was established from 26 healthy volunteers (23.6-33.8 seconds). Thus, aPTT values obtained with Pathromin SL were considered short if less than 23.6 seconds, normal if 23.6 to 33.8 seconds, and prolonged if more than 33.8 seconds. All analyses were run in parallel, so samples were thawed only once.

The patients' Clinical history including diagnoses was obtained from the Medical Records Department database. Diagnoses were categorized into following groups, formed according to the frequency of diagnoses observed: malignancy of Oral mucosa, Lung, Cervix, Breast, Lymphoreticular systems. Gastrointestinal systems, Ovary, Hematopoietic Systems, Bleeding and Trauma. Patients were contacted 6 to 12 months after the initial blood collection by questionnaire and follow-up interview. They were asked about any thromboembolic event during the previous 18 months.

To analyze the data, we compared variables stratified according to the aPTT (short vs normal). Statistical analysis (Mann-Whitney rank sum test, *t* test, *z* test, simple and multiple logistic

ORIGINAL ARTICLE

regression analysis, Kolmogorov-Smirnoff test) was performed using SigmaStat Software (Jandel Scientific, San Rafael, CA).

RESULTS: A total of 250 specimens were randomly collected according to the aPTT, as defined in the methods section; 131 specimens showed a short aPTT, and 119 specimens had a normal aPTT. Since samples were screened solely on the basis of their aPTT values, 12 patients (total of 22 specimens) were enrolled more than once, and, thus, 228 patients were enrolled. For the calculation of rates of thromboembolic events and diagnoses, patients were considered only once. At the time of data collection and analysis, final diagnoses and other patient information were available from the Medical Records Department for 225 of 228 patients. F1+2 levels were measured in 242 specimens because there was insufficient plasma left in 8 specimens.

An overview of the results is given in Table 1 and significantly more women were found in the short aPTT group. In reviewing our reference ranges, we found no significant sex difference between reference ranges using the Mann-Whitney U test ($P = .56$). Median F1+2 values were clearly and significantly higher in the short aPTT group; this difference was also present when only the first F1+2 value from patients enrolled more than once was considered (2.69 vs 1.47 nmol/L; $P < .001$).

Patients with a short aPTT had significantly more thromboembolic events (odds ratio, 2.38; 95% confidence interval, 1.07-6.10), namely, venous thromboses (odds ratio, 5.40; 95% confidence interval, 1.14-25.59) during the 18 months proximate to the blood collection. Unexpected bleeding episodes also were significantly more frequent in patients with a short aPTT. In addition, there was a strong trend toward more patients having a diagnosis of trauma in the group with a short aPTT in a multiple logistic regression analysis, a short aPTT was not significantly related to any of the specified diagnoses at hospitalization.

Thirteen patients in the short aPTT group had a diagnosis of bleeding with hospitalization; 11 of these patients participated in the questionnaire and follow-up. Of these 11 patients, 4 (36%) had thromboembolic events during the 18 months proximate to the time of the blood collection (venous thromboses, 3; stroke, 1).

With the Pathromtin SL reagent, 201 of the 239 specimens were normal or short. Of the 201 specimens, 24 were from patients enrolled more than once; of these 177 patients, only 118 participated in the questionnaire and follow-up. According to the reference range established for Pathromtin SL, 36 of the specimens demonstrated a short aPTT, 165 a normal aPTT, and 38 a prolonged aPTT. The 239 values were normally distributed (Kolmogorov-Smirnoff). Most findings made with the routine aPTT reagent were confirmed with this second reagent Table 2, although there were differences in age distribution and number of patients with diagnosis of trauma.

Multiple logistic regression analysis showed a significant relationship to a diagnosis of bleeding ($P = .011$); none of the other previously specified diagnoses at hospitalization showed significant relationship to a short Paratrombina PTT again showed clearly increased F1+2 levels, which was true if F1+2 values from patients with multiple enrollments were considered only once (3.53 vs 1.92 nmol/L; $P, 0.001$). similar to the findings with routine aPTT reagent, 2(405) of 5 patients hospitalized with some kind of bleeding episode and participating in the follow-up questionnaire also had a thromboembolic event during 18 months proximate to the blood collection (Venus thrombosis 1; stroke 1).

ORIGINAL ARTICLE

	Normal aPTT (27.9-31.9 s)	Short aPTT (<24.8 s)	P
Sex ratio (female/male)	52:59	72:42	.02(z)
Mean age \pm SD (y)	47 \pm 16	50 \pm 17	.13(t)
F1+2 (nmol/L), median	1.49	2.51	<.001(MW)
All thromboembolic events ^{1"}	9/70	20/73	.05(z)
Venous thromboses only	2/70	10/73	.04(z)
Bleeding*	2/111	13/114	.009(z)
Trauma*	6/111	16/114	.051(z)

Table 1: Results obtained with the Routine Activated Partial Thromboplastin Time(aPTT) Reagent*

z, z test; t, t test; MW, Mann Whitney rank sum test.

*Data are given as the number of patients with the diagnosis /total number of patients unless otherwise noted. Results are stratified into normal and short aP^ groups. All statistical comparisons are made between these 2 groups of patients.

^ The total number of patients is derived from all patients who responded to the questionnaire.

* The total number of patients derived from those with hospital chart diagnosis.

	Normal aPTT (23.6-33.8 s)	Short aPTT (<23.6 s)	P
Sex ratio (female/male)	85:66	23:7	.061(z)
Mean age \pm SD (y)	50 \pm 17	43 \pm 15	.026(t)
F1+2 (nmol/l), median	1.90	3.38	<.001 (MW)
All thromboembolic events ¹	14/99	8/19	.011(z)
Venous thromboses only ¹	5/99	4/99	.052 (z)
Bleeding*	6/147	5/30	.029 (z)
Trauma*	15/147	4/30	.856 (z)

Table 2: Results Obtained With the Control Activated Partial Thromboplastin Time (aPTT) Reagent*

z, z test; t, t test; MW, Mann Whitney rank sum test.

*Data are given as the number of patients with the diagnosis/total number of patients unless otherwise noted. Results are stratified into normal and short aPTT groups. All statistical comparisons are made between these 2 groups of patients.

^The total number of patients is derived from all patients who responded to the questionnaire.

*The total number of patients is derived from those with hospital chart diagnoses.

DISCUSSION: The aPTT has long been useful for predicting propensity for bleeding as is the case with Hemophilia and other congenital bleeding disorders⁽¹⁾. Consequently, in congenital bleeding states of Plasmatic origin. The aPTT tends to be prolonged with treatment of these congenital bleeding disorders, the prolonged aPTTs tends to shorten and correct to values near or within the normal aPTT reference range. In this instances the decrease in a prolonged aPTT is expression of the correction of a hypercoagulable state and, thus, is related to an increase in the procoagulant potential. In contrast with bleeding disorders and prolonged aPTTs, very little work exists on the possible meaning of aPTTs below lower limit of a reference or normal range. One large prospective trial evaluating this question concludes that short aPTTs probably representing increase in procoagulant potential⁽²⁾; however this conclusion has been disputed ⁽⁵⁾.

We have postulated that patients with aPTT values below the lower limit of normal range might have an increased procoagulant potential and thus an increase in thrombin generation. This hypothesis can be tested by measuring molecular markers of thrombin generation(eg.F1+2) in specimens with short and normal aPTTs. We prospectively selected routine blood samples sent to coagulation laboratory for aPTT testing if normal or short aPTT was present.

ORIGINAL ARTICLE

Our data show that a short aPTT indeed is related to an increase in thrombin generation, as substantiated by significantly increased F1+2 level. This indicates that aPTTs are sensitive not only to hypocoagulable state, but also to hypercoagulable state with increased thrombin formation. The clinical importance of this finding is underscored by the fact that patients with short aPTTs had more thrombotic events, predominantly venous thrombosis, during 18 month period before and after blood collection.

Interestingly we have found that significantly more patients in this group with short aPTT has a diagnosis of some type of bleeding during hospitalization. Such a relationship had been described twice.^(5, 6) These findings seemed to argue against the value of a short aPTT as a possible indicator for a hypercoagulable state. It is therefore exciting for us to find that 4(36%) of 11 patients with a short aPTT and bleeding episode during hospitalization also had thrombotic events during 18 months proximate to blood collection. These cases are important because they provide data supporting the 2 seemingly contradictory observation that a short aPTT is related to hypercoagulable state^(2, 4) as well as bleeding episodes.^(5, 6) We can only speculate on the mechanism of short aPTTs in our patients with a diagnosis of bleeding, but it seems reasonable to postulate that abnormally short aPTT with an increase in thrombin generation might be physiologic response of coagulation system to an abnormal blood loss. As suggested by Belliveau⁽⁵⁾ we have found evidence for such a scenario in patients undergoing surgery; those who had significantly more blood loss intraoperatively also had significantly higher markers of thrombin generation.⁽⁹⁾

In a prospective clinical trial, published in 1977, McKenna et al⁽³⁾ has evaluated the incidence of thromboembolic events assessed by chart review on discharge for 100 medical and surgical inpatients with a short aPTT. These investigators found presence of a short aPTT at any time during the study period to be related to a 10 fold increased incidence of thromboembolic events.

Other studies have supported the association of short aPTT with either thrombosis or hemorrhage, but unlike the present study, never both conditions in the same patient population. The studies of Landiet al⁽⁴⁾ and Gallus et al⁽¹¹⁾ suggested that a short aPTT was associated with an increased risk of thrombosis in specific clinical situations. Belliveau⁽⁵⁾ disputed the findings by McKenna et al⁽³⁾ and Gallus et al,⁽¹¹⁾ stating that in the patient population he evaluated, short aPTTs were related to bleeding rather than to thrombosis.

Gabazza et al⁽¹²⁾ reported that the aPTT became significantly shorter in a small patient population of patients with lung cancer after administration of chemotherapy. Maher et al⁽¹³⁾ reported evidence that decreases in the aPTT after simulated high altitude exposure to 4,000 m correlated with some rather mild and inconsistent elevation in fibrin degradation products. Finally, Vogt et al⁽¹⁴⁾ found a significant shortening of the aPTT in healthy volunteers after strenuous exercise, but with no apparent clinical sequelae.

The data of the present study demonstrate that patients with a short aPTT, which occurs in 6% of routine aPTTs, are at significantly increased risk for thromboembolism, mainly venous thromboses. Hypercoagulability is evidenced by an increase in thrombin generation. In the acute care setting, short aPTTs can be related to bleeding episodes, but in the present study, more than one third of these patients also had thromboembolism in the 18 months proximate to the blood collection.

REFERENCES:

1. White GC II, Marder VJ, Colman RW, et al. Approach to the bleeding patient. In: Colman RW, Hirsh J, Marder VJ, et al, eds. Hemostasis and Thrombosis: Basic Principles and Clinical

ORIGINAL ARTICLE

- Practice. 3rd ed. Philadelphia, PA: Lippincott; 1994: 1134-1147.
2. Feinstein DI. Immune coagulation disorders. In: Colman RW, Hirsh J, Marder VJ, et al, eds. Hemostasis and Thrombosis: Basic Principles and Clinical Practice. 3rd ed. Philadelphia, PA: Lippincott; 1994; 881-905.
 3. McKenna R, Bachmann F, Miro-Quesada M. Thromboembolism in patients with abnormally short activated thromboplastin time. *Thromb Haemost*. 1977; 38: 893-899.
 4. Landi G, D'Angelo A, Boccardi E, et al. Venous thromboembolism in acute stroke: prognostic importance of hypercoagulability. *Arch Neurol*. 1992; 49: 279-283.
 5. Belliveau RR. Extremely shortened activated partial thromboplastin times [letter]. *JAMA*. 1980; 243: 2286.
 6. Easa D. Coagulation abnormalities associated with localized hemorrhage in the neonate. *J Pediatr*. 1978; 92: 989-994.
 7. Aronson DL, Stevan L, Ball AP. Generation of the combined prothrombin activation peptide (F1+2) during the clotting of blood and plasma. *J Clin Invest*. 1977; 60: 1410-1418.
 8. Rosing J, Tans G. Meizothrombin, a major product of factor Xa-catalyzed prothrombin activation. *Thromb Haemost*. 1988; 60: 355-360.
 9. Korte W, Truttmann B, Heim C, et al. Preoperative values of molecular coagulation markers identify patients at low risk for intraoperative haemostatic disorders and excessive blood loss. *Clin Chem Lab Med*. 1998; 36: 235-240.
 10. Girolami A, Procidamo M, Vicariotto M, et al. The effect of low-dose estroprogestinic preparations on prothrombin complex factors: no significant increase after an 8-month trial. *Blut*. 1985; 50: 141-148.
 11. Gallus AS, Hirsh J, Gent M. Relevance of preoperative and postoperative blood tests to postoperative leg-vein thrombosis. *Lancet*. 1973; 2: 805-809.
 12. Gabazza EC, Taguchi O, Yamakami T, et al. Alteration of coagulation and fibrinolysis systems after multidrug anticancer therapy for lung cancer. *Eur J Cancer*. 1994; 30: 1276-1281.
 13. Maher JT, Levine PH, Cymerman A. Human coagulation abnormalities during acute exposure to hypobaric hypoxia. *J Appl Physiol*. 1976; 41: 702-707.
 14. Vogt A, Hoffmann V, Straub PW. Lack of Fibrin formation in exercise induced activation of coagulation, *Am J Physiol*. 1979; 226: H577-H579.

AUTHORS:

1. Indranil Dhar
2. Debkumar Ray
3. Debashis Roy Burman
4. Shibashish Bhattacharyya

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Laboratory Medicine, School of Tropical Medicine, Kolkata.
2. Assistant Professor, Department of Biochemistry, B. S. Medical College, Bankura, W.B.
3. Assistant Professor, Department of Laboratory Oncology (Oncopathology), Medical College, Kolkata.

4. Associate Professor, Department of Medical Oncology, Medical College, Kolkata.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Debkumar Ray,
KL-1, Ganga Yamuna Aptt,
Block-B, Flat- C & D,
Aswininagar, Kolkata – 700059.
Email: dr.debkumar@gmail.com

Date of Submission: 03/06/2014.
Date of Peer Review: 04/06/2014.
Date of Acceptance: 12/06/2014.
Date of Publishing: 19/06/2014.