

Assessment of activated protein C resistance and factor V Leiden in patients with thrombosis and cancer

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ABSTRACT

The relationship between cancer and thrombosis has long been known, but its functionality is poorly understood. In this study, we assess the prevalence of activated protein C resistance in patients with cancer and acute venous thromboembolism, comparing it with that of normal subjects and patients with venous thromboembolism but without cancer (83 patients with cancer, 101 without cancer, and 27 normal subjects). The mean activated protein C ratios were 3.38, 2.99, and 3.63, respectively; 16.9, 23.8, and 3.7%, respectively, had abnormal activated protein C ratios. Those patients with abnormal ratios were tested for the factor V Leiden mutation. Twenty-nine percent of patients with cancer and 55% of patients without cancer were heterozygotes for the factor V Leiden mutation. Our study suggests that activated protein C resistance has a role in the development of venous thromboembolism in cancer and, moreover, that there is a functional activated protein C resistance in persons with cancer, the etiology of which remains uncertain. *Lab Hematol* 5:70-73, 1999

KEY WORDS Activated protein C resistance · Cancer
· Factor V Leiden · Thrombosis

INTRODUCTION

The relationship between cancer and thrombosis has been known since 1865, when it was first described by Trousseau [1]. Hemostatic abnormalities are common in patients with malignancy, and about 15% of patients develop an episode of clinical thrombosis [2,3]. Known predisposing factors for venous thromboembolism

include the triad of venous stasis, injury, and hypercoagulability (Virchow's triad) [3]. Cancer itself has traditionally been considered a "hypercoagulable state," and many aspects about cancer are known or suspected to predispose patients to developing venous thrombosis [4,5].

Until recently, only up to 10% of patients with idiopathic venous thromboembolism were found to have one of the then-known hypercoagulable states such as protein C, protein S, or antithrombin III deficiency or lupus anticoagulant/anticardiolipin antibody [3]. In the last 5 years, two new hypercoagulable states have been described, one of which initially was called activated protein C (APC) resistance [6]; the other is an abnormality in the gene for prothrombin [7]. Most cases of APC resistance have been found to be due to the factor V Leiden mutation, which is a one-amino acid substitution in the gene for factor V [8,9]. This mutation is present in up to 6% of normal Caucasians without history of venous thromboembolism. Various case series have assessed the prevalence of APC resistance or the factor V Leiden mutation in patients with venous thromboembolism and found it to explain anywhere from 20 to 70% of cases, depending on the population studied [10].

Although venous thromboembolism is common in persons with cancer, most do not develop the often fatal complication. To date there has been no formal assessment of the prevalence of APC resistance or the factor V Leiden mutation in patients with cancer with concurrent symptomatic venous thromboembolism. In this study, we assess the prevalence of APC resistance in patients with cancer and acute venous thromboembolism, comparing it with that of normal subjects and persons with venous thromboembolism but without cancer.

METHODS

This prospective cohort study was performed at the London Health Sciences Centre, Victoria Campus, London, Ontario. We studied all patients with an objectively confirmed diagnosis of deep vein thrombosis or pulmonary embolus from March 1994 to

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TABLE 1. Comparison of APC resistance

	Thrombosis with cancer	Thrombosis without cancer	Normal subjects
n	83	101	27
Number with abnormal APC ratio (%)	14 (16.9)	24 (23.8)	1 (3.7)
APC ratio	3.38 ± 0.11	2.99 ± 0.06	3.63 ± 0.12

APC ratios are means ± SE.

December 1995. Patients were excluded for the following: a known history of protein C, protein S, or antithrombin III deficiency or presence of a lupus anticoagulant; acquired coagulopathy at diagnosis (activated partial thromboplastin time [aPTT] >40 seconds using IL-aPTT, a lupus-sensitive reagent, or an abnormal international normalized ratio [INR]); or age <18 years.

Demographics

Patients were divided into three groups as follows: 1) thrombosis with no history of active cancer; 2) thrombosis with active cancer; 3) healthy, normal volunteers with no history of thrombosis. A careful chart review was performed to ensure inclusion/exclusion criteria and verify patient group assignment (cancer or no cancer).

Activated Protein C Resistance Testing

Whole blood was collected into vacutainer tubes (Becton Dickinson, Rutherford, NJ) containing 3.8% citrate (before initiation of warfarin). Samples were double-spun at 1700g, and plasma was frozen at -70°C for later batch testing.

Samples were processed on an ACL 300+ (Instrumentation Laboratory; Coulter Electronics, Miami, FL) using Coatest APC Resistance (Chromogenix, Mölndal, Sweden). Plasma was incubated with the aPTT reagent for a standard period of time, coagulation was triggered by the addition of calcium chloride in the absence and presence of activated protein C, and the time for clot formation was recorded. The ratio of clot time with APC/CaCl divided by clot time with CaCl was calculated. The testing system was monitored by commercial controls, control plasma level 1 (2.6–4.2) and level 2 (1.5–2.4) provided by Chromogenix. The normal range was verified for the reagent/instrument combination. Abnormal samples were defined as having ratios <2.6.

Factor V Leiden Testing

The relevant region of the factor V gene was amplified from leukocyte-derived genomic DNA by polymerase chain reaction (PCR) with primers flanking the mutant site, whose presence could be inferred by the loss of an *MnlI* restriction enzyme cut site. The 246-bp DNA generated by PCR was divided by *MnlI* digestion into three fragments from the normal allele (37, 48, and 161 bp) and two fragments (85 and 161 bp) from the mutant allele. These DNA fragments were sized by polyacrylamide gel electrophoresis, and DNA was visualized by staining with ethidium bromide. The constant *MnlI* digestion site acted as an internal control.

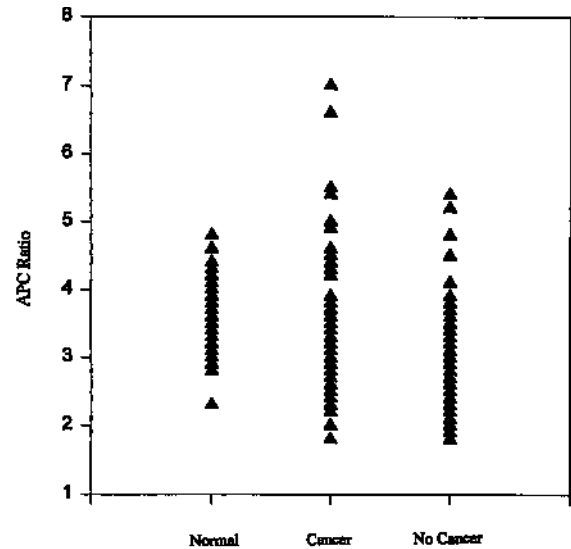


FIGURE 1. Distribution of activated protein resistance.

Analysis

APC resistance testing was done on all patient samples. If the APC ratio was abnormal (<2.6), then confirmatory testing for the factor V Leiden mutation was performed. The groups were compared for 1) proportion of patients with abnormal APC resistance (<2.6); 2) mean APC resistance; and 3) proportion of patients with abnormal APC resistance due to the presence of the factor V Leiden mutation.

Statistical Analysis

The three groups were compared for the proportion of abnormal APC resistance using Pearson χ^2 . The mean APC ratios were compared by analysis of variance (ANOVA) and Bonferroni multiple comparisons. Finally, the proportion of abnormal APC resistance due to the factor V Leiden mutation was compared using Pearson χ^2 .

RESULTS

Table 1 compares the three groups for the number with an abnormal APC ratio and for the mean APC ratio. The distribution of the ratios for the three groups is illustrated in Figure 1. Using Pearson χ^2 , the groups were not significantly different with respect to the proportion of patients with abnormal APC ratio ($p = 0.18$). The three groups were significantly different with respect to the mean APC ratio by ANOVA at the 5% level of significance. By Bonferroni analysis, there was no difference between cancer patients and normal subjects. There was a significant difference between the patients with and without cancer (95% confidence interval [CI] 0.11–0.67) and between the patients without cancer and normal subjects (95% CI 0.23–1.05).

As shown in Table 2, 20 of 24 patients without cancer with an abnormal APC ratio and all cancer patients with an abnormal APC ratio were assessed for the factor V Leiden mutation. The proportion of abnormal APC ratios due to the factor V Leiden mutation for patients with cancer was 29% vs. 55% for patients without cancer;

TABLE 2. Testing for factor V Leiden mutation

	Patients with cancer	Patients without cancer
Number with positive APC ratio	14	24
Number tested for factor V Leiden	14	20
Number heterozygous for factor V Leiden (%)	4 (29)	11 (55)

the difference approached statistical significance by χ^2 ($p = 0.127$). Overall, only 4.8% of patients with cancer and thrombosis were found to have the factor V Leiden mutation.

DISCUSSION

Until recently, very few patients who presented with venous thromboembolism were found to have a genetic abnormality predisposing them to this condition [3]. Recently, two newly discovered genetic defects, specifically the factor V Leiden mutation and prothrombin 20210A, have increased that number substantially [6,7].

Patients with active cancer have long been known to have a high risk of venous thromboembolism. Although there are many hypotheses as to the mechanism of this effect, it remains uncertain [4,5]. In this study, we wished to assess if there was evidence that APC resistance and the factor V Leiden mutation could account for the increased incidence of thrombosis in cancer patients.

Of the three groups in our study, the first two all had acute, objectively documented deep vein thrombosis or pulmonary embolus and were subdivided by the presence or absence of active cancer. The third group consisted of normal volunteers without history of cancer or thrombosis. Our study found overall clinically significant—but not statistically significant—differences between the three groups with respect to the proportion of persons with an abnormal APC ratio. The lack of statistical significance was likely due to inadequate sample numbers. When the groups were compared for the mean population APC ratio itself, both of the venous thromboembolism groups had ratios lower than those of normal subjects, although only those without cancer were significantly different. Finally, we assessed the proportion of patients with a “true” abnormal APC ratio (due to confirmed factor V Leiden mutation) between the groups with and without cancer. Seventy-one percent of patients with cancer had a “false” abnormal APC ratio, compared with only 45% of those without cancer. The results only approached statistical significance, again likely because of insufficient numbers of patients. Only 4.8% of the cancer patients overall were found to have the factor V Leiden mutation.

Our findings, although hampered by inadequate sample size, are consistent with those of others. The initial commercially available screening test for APC resistance (the one employed in this study) has been shown to have numerous false normals [11]. Other conditions that have been shown to lower the APC ratio include pregnancy [12], use of birth control pills [13], high factor VIII levels [14], and cancer itself [15]. As a result of these problems, the tests have been modified [16]. By using factor V-deficient plasma con-

trols, newer APC screening tests are much more specific for the factor V Leiden abnormality than the test employed in this study [11], which was conducted before the factor V-deficient plasma test kits were available. Despite these findings, a recent study has shown that APC resistance is a risk factor for thrombosis independent of factor V Leiden [17].

As far as we know, our study is the first assessment of APC resistance in patients with cancer and thrombosis. Our results suggest that APC resistance has a role in the development of thrombosis in patients with active cancer. Moreover, there appears to be functional APC resistance with cancer similar to that previously described in other conditions, and further studies seem warranted. Functional APC resistance may be a contributing factor to the increased risk of thrombosis in persons with cancer; however, the mechanism of this remains uncertain. The ultimate goal would be to identify specific laboratory abnormalities in patients with cancer to indicate those that are at higher risk of developing deep vein thrombosis or pulmonary embolus. Those patients would potentially be candidates for primary prophylaxis.

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