

## Venous Limb Gangrene during Warfarin Treatment of Cancer-Associated Deep Venous Thrombosis

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**Background:** The cause of cancer-associated venous limb gangrene is unknown but could paradoxically be due to warfarin.

**Objective:** To determine the pathogenesis of venous gangrene in a patient with cancer.

**Design:** Case report.

**Setting:** University hospital in Ontario, Canada.

**Patient:** 66-year-old woman with metastatic lung cancer and deep venous thrombosis.

**Measurements:** Levels of vitamin K–dependent factors, additional coagulation factors, and thrombin–antithrombin complexes (marker of thrombin generation).

**Results:** During warfarin use, venous limb gangrene developed

when the international normalized ratio (INR) reached 6.0 (therapeutic range, 2.0 to 3.0); at this time, the level of protein C (a vitamin K–dependent natural anticoagulant) was severely reduced, but thrombin–antithrombin complexes remained markedly elevated. The supratherapeutic INR was explained by the greatly reduced levels of factor VII, which correlated closely with protein C levels; therefore, the high INR was a surrogate marker for severely reduced protein C activity.

**Conclusion:** Warfarin may contribute to the pathogenesis of cancer-associated venous limb gangrene by leading to severe depletion of protein C while at the same time failing to reduce thrombin generation.

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Venous limb gangrene is characterized by progression of deep venous thrombosis to limb necrosis despite palpable or Doppler-identifiable arterial pulses. Although this disorder can occur in patients with metastatic cancer (1–3) or heparin-induced thrombocytopenia (4–6), the pathogenesis is obscure. Venous gangrene complicating heparin-induced thrombocytopenia may be associated with oral anticoagulant (coumarin) therapy, including warfarin (4, 5) or phenprocoumon (6). Coagulation factor studies in such patients during warfarin use show persistence of thrombin generation despite warfarin therapy but severe depletion in protein C, which is a vitamin K–dependent natural anticoagulant (4). The availability of serial plasma samples from a patient with metastatic cancer who developed warfarin-associated venous limb gangrene provided an opportunity to study the pathogenesis of this syndrome in a patient with cancer-associated hypercoagulability.

### CASE REPORT

A 66-year-old woman with metastatic lung carcinoma developed right femoral deep venous thrombosis and received heparin and warfarin. Because the platelet count increased from 328 to  $682 \times 10^9$  cells/L during heparin therapy but decreased to  $121 \times 10^9$  cells/L after heparin therapy was stopped, heparin-induced thrombo-

cytopenia was suspected (Figure 1, top). Although the international normalized ratio (INR) increased to 7.2 (therapeutic range, 2.0 to 3.0), a new episode of left femoral deep venous thrombosis developed. Warfarin therapy was resumed when the INR decreased to 2.1. However, the INR then increased to 6.0, and swelling, cyanosis, and pain in the left leg developed (phlegmasia cerulea dolens). This condition progressed to necrosis of the toes and foot despite palpable pedal pulses, and the patient died the next day of respiratory failure.

### METHODS

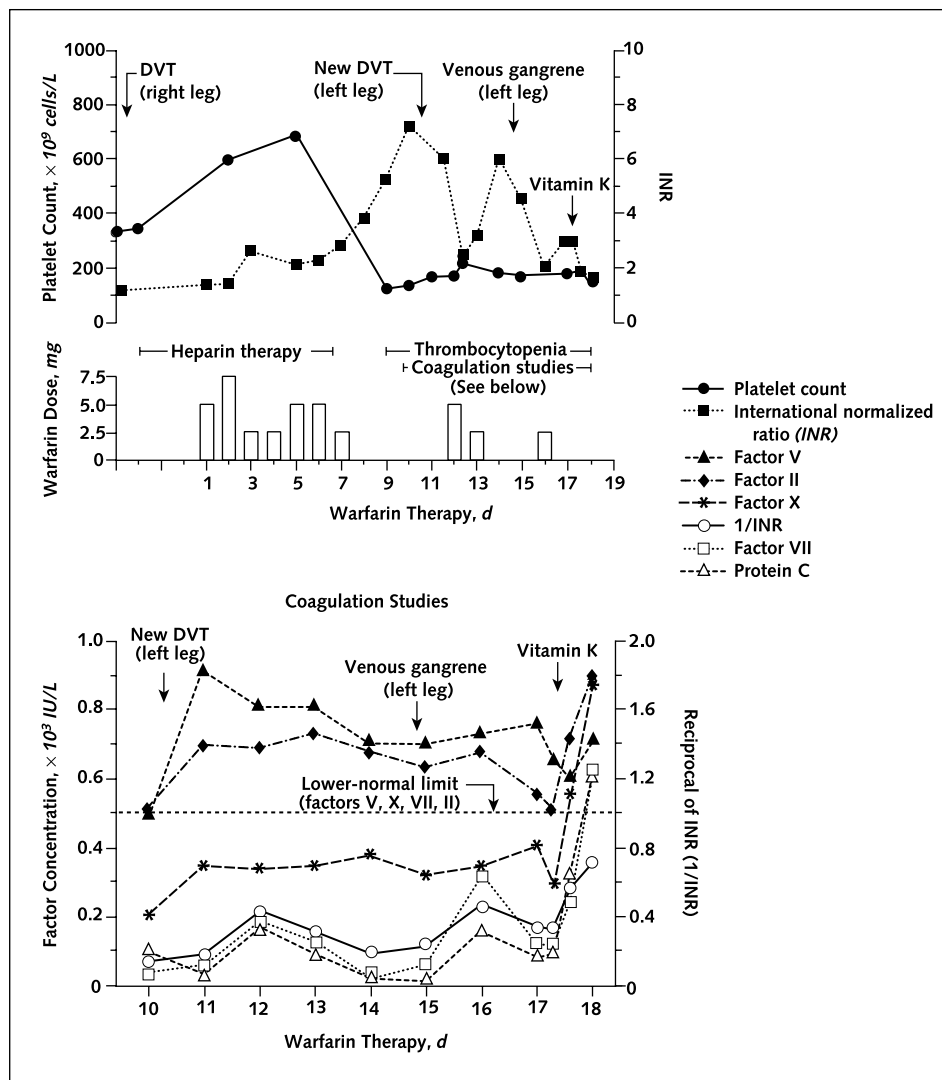
#### Plasma Sample Collection

Plasma samples were collected as part of an ethics review board–approved study of patients with suspected heparin-induced thrombocytopenia (4). Eleven plasma samples from the patient had been obtained during days 10 to 18 of warfarin treatment. The comparison group consisted of 34 consecutive patients with serologically confirmed heparin-induced thrombocytopenia, of whom 20 had received warfarin therapy (4 of these patients developed venous gangrene), as well as 59 patients with postoperative deep venous thrombosis (4).

#### Laboratory Studies

International normalized ratios were determined by using an ACL model 300 instrument (Instrumentation

Figure 1. Venous limb gangrene complicating deep venous thrombosis (DVT) and cancer.



**Top.** Serial platelet counts and international normalized ratio (INR) values in relation to heparin and warfarin treatments. The abrupt increase in the INR and decrease in the platelet counts on discontinuation of heparin therapy are consistent with the increase in the consumption of coagulation factors and platelets in this patient with cancer-associated disseminated intravascular coagulation resistant to warfarin. **Bottom.** Plasma factor studies. Eleven plasma samples obtained between days 10 and 18 of warfarin treatment were studied. Vitamin K, 5 mg intravenously, was given where indicated. Of the four coagulation factors that affect the INR (factors II, V, VII, and X), only factor VII levels explain the variability in the reciprocal of the INR:  $1/\text{INR} = 0.178 + 0.974[\text{factor VII level}]$  ( $r^2 = 0.883$ ;  $P < 0.001$ ). Protein C activity showed a near-identity relationship with factor VII:  $[\text{protein C level}] = -0.01 + 0.94[\text{factor VII level}]$ . The following coagulation factor levels (not shown in the figure) were normal and are expressed as the mean ( $\pm$ SD) (normal range,  $0.50$  to  $1.50 \times 10^3$  IU/L, except where noted): factor VIII,  $2.87 \pm 1.37 \times 10^3$  IU/L; factor IX,  $0.81 \pm 0.34 \times 10^3$  IU/L; factor XI,  $0.69 \pm 0.12 \times 10^3$  IU/L; factor XII,  $0.54 \pm 0.09 \times 10^3$  IU/L; free protein S,  $125 \pm 18$  nmol/L (normal range, 86 to 221 nmol/L); fibrinogen,  $2.67 \pm 0.55$  g/L (normal range, 1.5 to 4 g/L); and antithrombin activity,  $1.03 \pm 0.06 \times 10^3$  IU/L (normal range, 0.77 to  $1.30 \times 10^3$  IU/L).

Laboratories, Milan, Italy) and human placental thromboplastin (Thromborel, Dade Behring Diagnostics, Marburg, Germany). Vitamin K-dependent factors (II, VII, IX, and X) and other coagulation factors (V, VIII, XI, and XII) were measured by using clotting assays.

Protein C activity, free protein S, antithrombin, and thrombin-antithrombin complex levels were measured, as described elsewhere (4). The platelet  $^{14}\text{C}$ -serotonin release assay and the platelet factor 4-heparin-immunoassay were used to detect heparin-dependent antibodies (7).

### Statistical Analysis

Multiple linear regression analysis was done on data from all 11 plasma samples by using Minitab software, release 13.31 (2001) (Minitab, Inc., State College, Pennsylvania), to assess the relative strengths of the four coagulation factors that influence the INR (factors II, V, VII, and X) in predicting the INR and its reciprocal. (Lind and colleagues [8] found that the vitamin K–dependent factor concentrations are related linearly to 1/INR.) Variables were entered into the model in a stepwise fashion. To check the potential independence violation caused by use of repeated measurements from one patient, the Durbin–Watson statistic (9), which tests the residuals for first-order autoregressive correlation, was calculated, and its statistical significance was assessed.

### Role of the Funding Source

The funding source had no role in the collection, analysis, or interpretation of the data or in the decision to submit the report for publication.

### RESULTS

Serum samples obtained from the patient between days 11 and 18 of warfarin therapy tested negative for heparin-dependent antibodies on the platelet  $^{14}\text{C}$ -serotonin release assay and platelet factor 4–heparin-immunoassay.

Thrombin–antithrombin complex levels were greatly elevated in the patient’s 11 plasma samples (median, 66  $\mu\text{g/L}$  [range, 33 to 110  $\mu\text{g/L}$ ] [normal, <4  $\mu\text{g/L}$ ]). The highest thrombin–antithrombin complex level occurred at the onset of limb necrosis, even though the INR measured 6.0. All thrombin–antithrombin complex levels in the patient were higher than those of the 59 controls who had postoperative deep venous thrombosis (median, 7.6  $\mu\text{g/L}$  [range, 1.9 to 26.0  $\mu\text{g/L}$ ]) but were similar to levels in patients with heparin-induced thrombocytopenia (median, 43.2  $\mu\text{g/L}$  [range, 2.1 to 225  $\mu\text{g/L}$ ]) (4).

The bottom panel of **Figure 1** shows levels of the three vitamin K–dependent factors (II, VII, and X) and the non–vitamin K–dependent factor (V) known to influence the INR. Despite INR values as high as 7.2, prothrombin (factor II) levels remained in the normal range, and factor X levels were only moderately decreased. In contrast, factor VII levels were severely reduced. Linear regression analysis showed that factor VII

activity best predicted the reciprocal of the INR ( $P < 0.001$ ) and explained 88.3% of the total variability in the reciprocal of the INR; factors X, II, and V showed no statistically significant additional influence. No substantial serial autocorrelation was seen in the modeling according to the Durbin–Watson test statistic ( $P > 0.05$ ).

Protein C activity levels correlated closely with factor VII levels ( $r = 0.94$ ;  $P < 0.001$ ) (**Figure 1, bottom**). Protein C levels reached their nadir ( $0.01 \times 10^3$  IU/L) as the deep venous thrombosis progressed to venous gangrene. Protein C activity increased to a near-normal level after administration of vitamin K.

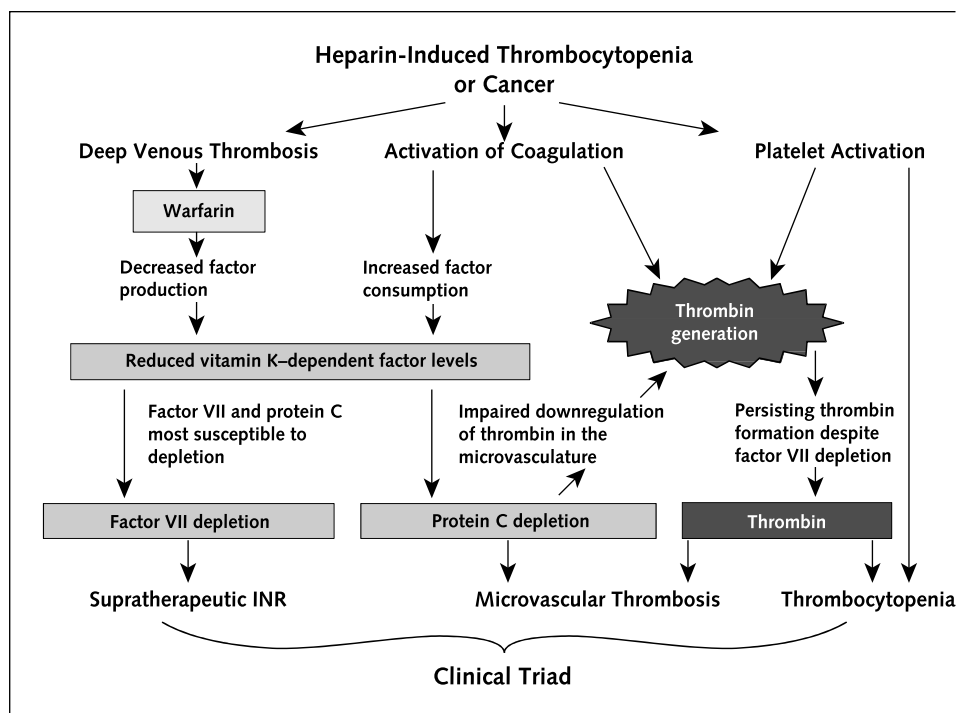
### DISCUSSION

A patient with cancer-associated deep venous thrombosis developed venous limb gangrene during warfarin therapy. Certain features in this case resemble the clinical triad of venous gangrene reported in patients with heparin-induced thrombocytopenia (4, 5). First, progression to limb necrosis occurred despite palpable pedal pulses, a finding consistent with microvascular thrombosis. Second, onset of necrosis coincided paradoxically with warfarin treatment that caused a supratherapeutic INR. Finally, the patient had thrombocytopenia.

However, heparin-dependent antibodies were not detected in this patient. Instead, the increasing platelet count during heparin therapy, the abrupt decrease in platelet count on stopping of heparin therapy, and the development of a new episode of venous thrombosis during warfarin therapy were consistent with a cancer-associated, thrombin-mediated, platelet-consumptive state (disseminated intravascular coagulation) controlled by heparin but “resistant” to warfarin (10). The failure of warfarin to control thrombin-mediated hypercoagulability was confirmed by greatly elevated thrombin–antithrombin complex levels that persisted despite an increase in the INR to 7.2—even as warfarin led to severe depletion of two vitamin K–dependent factors, protein C and factor VII. The high ratio of thrombin–antithrombin complexes to protein C activity resembles that seen in patients with warfarin-associated venous gangrene complicating heparin-induced thrombocytopenia (4).

Protein C is converted to activated protein C by thrombin bound to thrombomodulin on microvascular endothelium (11). Activated protein C downregulates thrombin generation by proteolyzing factors Va and VIIIa; therefore, severely reduced protein C levels in a

**Figure 2.** Pathogenesis of warfarin-associated venous limb gangrene complicating heparin-induced thrombocytopenia or cancer.



The pathogenesis of warfarin-associated venous limb gangrene is shown in relation to its typical clinical triad—supratherapeutic international normalized ratio (*INR*), microvascular thrombosis, and thrombocytopenia. The central paradox is persisting formation of thrombin despite markedly depleted plasma factor VII level, which is paralleled by severely depleted protein C activity, leading to impaired downregulation of thrombin generation in the microvasculature and, consequently, microvascular thrombosis.

patient with poorly controlled thrombin generation secondary to heparin-induced thrombocytopenia or cancer could lead to microvascular thrombosis (4).

Analysis of serial plasma samples from this patient provides insight into the paradox of progressive thrombosis despite a supratherapeutic *INR*. Multiple linear regression analysis showed that almost all the variability in *INR* was explained by changes in factor VII levels. This was unexpected, as reduced factor II and factor X levels usually also substantially affect the *INR* in warfarin-treated patients (8). Nevertheless, despite the elevated *INR* and corresponding severe reduction in factor VII, warfarin failed to control the hypercoagulable state.

The paradox is further clarified by the near-identity relationship observed between protein C and factor VII levels (Figure 1, bottom). In effect, the supratherapeutic *INR* represented a surrogate marker for severe acquired protein C deficiency. Certain characteristics of factor VII and protein C could account for their greater sus-

ceptibility to depletion during warfarin therapy: The physiologic half-lives (5 hours for factor VII and 9 hours for protein C) are much shorter than those of the other vitamin K–dependent factors (factor II, 60 to 100 hours; factor X, 40 hours; factor IX, 24 hours) (12), making them especially susceptible to depletion during early warfarin therapy (13). In addition, the plasma concentrations of factor VII and protein C (10 nmol/L and 60 nmol/L, respectively) are lower than those of the other factors (factor IX, 90 nmol/L; factor X, 140 nmol/L; factor II, 1400 nmol/L), suggesting they may also be more vulnerable to depletion when coagulation factor consumption is pathologically increased because of cancer or heparin-induced thrombocytopenia.

Figure 2 summarizes the proposed pathogenesis of warfarin-associated venous limb gangrene complicating cancer or heparin-induced thrombocytopenia. Both disorders are associated with deep venous thrombosis (14, 15) and pathologic activation of hemostasis by proco-

agulant (16, 17) and platelet-activating factors (18, 19). These pathologic effects may explain thrombin generation that persists even when factor VII levels are very low.

Heparin is often effective for cancer-associated thrombosis that is resistant to warfarin (10). This is probably related to the different action of heparin, which catalyzes inactivation of several coagulation enzymes (thrombin, factor Xa, factor IXa, and factor VIIa–tissue factor [20]), whereas warfarin alters synthesis of the corresponding inactive precursors. Alternative anticoagulants that inhibit factor Xa (danaparoid) or thrombin (argatroban, lepirudin) can be used pending exclusion of heparin-induced thrombocytopenia (6).

This report of a patient with cancer and a previous study (4) of patients with heparin-induced thrombocytopenia suggest that warfarin can contribute to the pathogenesis of venous limb gangrene. The paradoxical role of warfarin may not always be recognized, as this complication might primarily be attributed to the underlying disease (1–3). However, **Figure 2** indicates the importance of both a predisposing hypercoagulable state (heparin-induced thrombocytopenia or cancer) and warfarin use. Thus, venous limb gangrene reflects a profound disturbance in procoagulant–anticoagulant balance, in which warfarin fails to control thrombin generation caused by the underlying disease, while at the same time it predisposes to microvascular thrombosis by severely reducing the level of protein C, a vitamin K–dependent natural anticoagulant.

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