# Laboratory assays and duplex scanning outcomes after symptomatic deep vein thrombosis: Preliminary results

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*Purpose:* The purpose of this article was to assess a number of hematologic and fibrinolytic assays at the time of diagnosis of deep vein thrombosis (DVT) and at several intervals over a period of 6 months afterward and to correlate these results with the results of serial duplex scanning.

*Methods:* Thirty-five patients (average age 61, range 18 to 82) with acute symptomatic DVT confirmed by duplex scanning were included. On diagnosis, blood was drawn, and plasma levels of tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor (PAI), D-dimer (DD), and tissue factor pathway inhibitor (TFPI) were determined. Duplex scanning and all laboratory assays were repeated 1 week, 1 month, 3 months, and 6 months thereafter.

*Results:* The rate of DVT complete resolution 6 months after diagnosis was 57%. Whereas plasma levels of PAI were similar throughout the 6-month follow-up period, t-PA increased significantly 1 week after diagnosis and decreased thereafter. Both DD and TFPI levels decreased significantly after diagnosis compared with presentation values. Comparing these assay levels between patients with complete resolution versus partial or no resolution, PAI levels were significantly higher during the first week in patients with poor outcome. Plasma levels of t-PA were higher in cases with good outcome, and DD levels were higher in patients with poor outcome. TFPI levels were similar in both outcome groups.

*Conclusions:* Patients with complete DVT resolution on duplex scanning at 6 months had significantly lower levels of PAI on presentation and after 1 week than did those with incomplete lysis. Although differences were not significant, t-PA levels were higher and DD lower in patients with good outcome. Our results suggest that certain plasma fibrinolytic assays might correlate with the outcome of DVT, as assessed by duplex ultrasonography. (J VASC SURG 1996;23:616-21.)

The natural history of deep vein thrombosis (DVT) is not fully understood. The final outcome of DVT depends on a balance between factors that promote further clot propagation or lead to its removal.[1] Thrombus propagation is facilitated by

factors such as stasis, hypercoagulability, or vascular damage. On the other hand, a clot may be partially or completely removed by fibrinolytic enzymes or become organized and eventually recanalized by leukocytes, fibroblasts, and capillary proliferation.[2]

A number of investigators have assessed the fate of venous thrombi of the legs for several weeks after diagnosis by means of B-mode ultrasonography or duplex scanning.[3-7] Most of these studies have demonstrated that more than 50% of vein thrombi undergo complete resolution 3 to 6 months after diagnosis. Some reports indicate that thrombi located distal to the knee joint are more easily lysed,[8] whereas others showed faster resolution in proximal thrombi.[9] Yet other studies could not find significant differences in the rates of thrombus resolution among different

vein segments of the legs.[3,7] Other factors such as age of the thrombus, clot extension, thrombus composition, and amount of enzymatic activity have been suggested to influence thrombus outcome.[10]

A few studies have investigated the effect of anticoagulant therapy on a number of plasma markers of hemostatic activation and fibrinolysis in patients with documented DVT, with controversial results.[11-13] In this study, we prospectively assessed the evolution of plasma levels of plasminogen activator inhibitor (PAI), tissue-type plasminogen activator (t-PA), D-dimer (DD), and tissue factor pathway inhibitor (TFPI) at the time of DVT diagnosis and then at different intervals for 6 months. In addition, we tried to establish correlations between these plasma markers and long-term DVT outcome, as assessed by serial duplex scanning.

## METHODS

Thirty-five patients (24 men and 11 women) referred to the vascular laboratory of our hospital with suspected DVT, in whom this diagnosis was confirmed, were included in the study. The protocol and consent form were approved by our hospital institutional review board. Average age of the patients was 61 years (range 18 to 82). All patients were initially admitted with physical signs and symptoms suggestive of DVT, that is, leg pain, tenderness, and swelling. Two patients had bilateral thrombi. Accordingly, there were 37 legs with DVT confirmed by duplex ultrasonography. In addition, 11 patients had confirmed pulmonary emboli, as detected by lung scanning. Twelve patients (34%) were already receiving intravenous heparin at the time of duplex examination.

All patients received standard intravenous heparin treatment, given by continuous infusion, adjusted to prolong the activated partial thromboplastin time between 2.0 to 2.5 times the control. Oral anticoagulants were started after patients had received heparin for 5 to 10 days, at doses adjusted to target an international normalized ratio between 2.5 and 3, at least for 3 months.

Duplex examinations were performed with an Ultramark 9 HDI color scanner (Advanced Technology Laboratories, Bothell, Wash.) on presentation and 1, 4, 12, and 24 weeks thereafter. Patients were placed in a 10- to 20-degree reverse Trendelenburg position to examine common femoral and superficial femoral veins. Popliteal, posterior tibial, and the remaining calf veins were studied with the leg in a dependent position. Images of all veins were obtained with transverse and longitudinal views. Abnormal

criteria, indicative of DVT, included absence of both spontaneous and augmented Doppler signal, presence of intraluminal echogenic filled material, inability to compress vein with gentle probe pressure, and dilation of the thrombosed vein. The percentage of obstruction of the vein lumen caused by the thrombus was calculated for each vein segment.

Regarding DVT outcome, worsening was reported if the clot extended to vein segments previously unaffected. No change indicated the presence of an unaltered clot. Improvement was reported if partial recanalization was evident, with reduction in the clot size, but with persistence of incomplete vein compression. A clot was considered to have resolved when the above-mentioned DVT criteria had disappeared.

Blood was gently drawn early in the morning by a double syringe technique and collected in citrated Vacutainer (Becton Dickinson; Franklin Lakes, N.J.) tubes and centrifuged at 2000 $g$ for 15 minutes at room temperature. Aliquots of plasma were stored at −70° C for later use. The following enzyme immunoassays were performed: PAI-1 (Biopool International, Ventura, Calif.), t-PA (Biopool), DD (Diagnostica Stago; Asnieref, France), and TFPI (American Diagnostica, Greenwich, Conn.). All four assays were performed at the time of DVT diagnosis confirmation and repeated 1, 4, 12, and 24 weeks thereafter.

The values of the four assays were found to be normally distributed. Statistical analysis consisted of calculating the mean and standard deviation. Paired $t$ testing was done for within-subject comparison of the blood tests at different time intervals compared to baseline. Independent Student $t$ testing was used to compare the blood assays results, depending on the outcome, at each time. The correlations between markers and age and among them were calculated by a linear regression model. Proportions were compared by the Fisher exact test. All tests were two-sided, and probability level of 0.05 or less was considered significant. These tests were carried out by use of the SPSS-PC software (SPSS Inc, Chicago, Ill.) run on an 486DX2 IBM-compatible computer (Packard-Bell; Chatsworth, Calif.).

## RESULTS

On presentation, duplex scanning identified 19 thrombi (51%) with 100% obstruction of the vein lumen. Proximal DVT, defined as *above-knee joint*, was reported in 29 patients (78%).

The results of duplex examination, with regard to DVT outcome, are presented in Table I. There was a gradual improvement of the thrombotic process
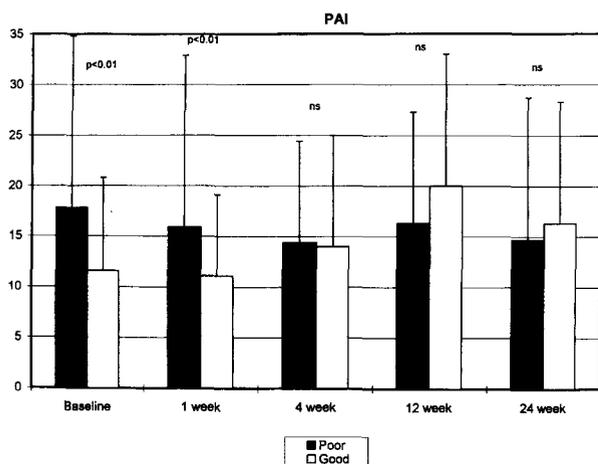
**Fig. 1.** Evolution of PAI-1 levels during 6-month follow-up period in patients undergoing complete DVT resolution ("good") and in those who underwent incomplete or no thrombus resolution ("poor"). Values expressed as mean and SD (ng/ml). Comparison between two outcome groups at each time interval means of independent *t* testing.
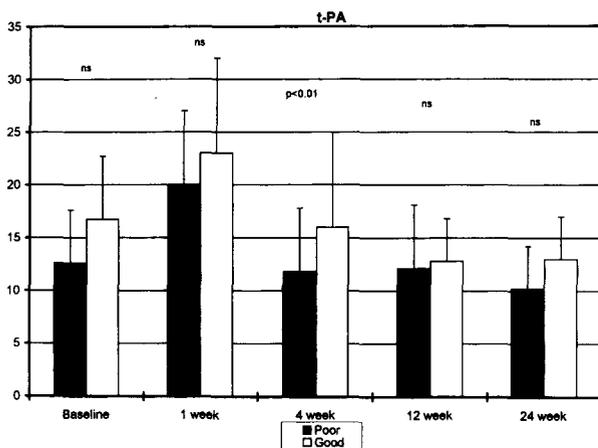


**Fig. 2.** Evolution of t-PA in patients admitted with DVT (See explanation in Fig. 1). Values expressed as mean and SD (ng/ml). Independent *t* testing.

throughout the study period. Six months after diagnosis most thrombi had either resolved (57%) or improved (20%). On the contrary, 14% remained the same, and 8% had worsened.

Patients were divided into two groups, depending on the results of duplex scanning obtained at 6 months: (1) poor outcome, which means incomplete resolution (43%, 15 cases), and (2) good outcome, which indicates complete resolution (57%, 20 cases). There was not a statistically different outcome depending on the level of thrombosis on presentation

**Table I.** DVT outcome at different time intervals

| Outcome | 1 week | 4 weeks | 12 weeks | 24 weeks |
|---|---|---|---|---|
| Worse | 3 (9%) | 1 (3%) | 2 (6%) | 3 (9%) |
| No change | 15 (43%) | 9 (26%) | 5 (14%) | 5 (14%) |
| Improvement | 16 (45%) | 23 (66%) | 21 (60%) | 7 (20%) |
| Resolved | 1 (3%) | 2 (6%) | 7 (20%) | 20 (57%) |

($p = 0.97$) or the presence of complete obstruction of the vein lumen ($p = 0.23$).

There was not a statistically significant correlation between the administration of heparin at the time of the baseline duplex examination and the outcome, because 64% of patients who were not given heparin at the time of diagnosis had good outcome, as compared with 54% in those receiving heparin ($p = 0.39$). On the other hand, 35% of patients receiving heparin on diagnosis had obstructive DVT, as compared to 77% of those who did not receive heparin ($p < 0.01$). A correct level of early anticoagulation (2 × control partial thromboplastin time) was achieved in 57% of patients. In this group of patients successfully treated with anticoagulation, 65% of cases had good outcome, compared to 31% of patients with treated inappropriate anticoagulation who had good outcome ($p = 0.06$). We do not have enough data regarding international normalized ratio levels after hospital discharge to correlate with final DVT outcome.

Overall results of the plasma assays, irrespective of the DVT outcome, are presented in Table II. Except for PAI, all the other marker levels experienced significant differences during the follow-up determinations compared to baseline.

A comparison of PAI levels in both outcome groups is shown in Fig. 1. Both on baseline and 1 week after DVT diagnosis, PAI levels were significantly higher in cases with poor final outcome.

The t-PA levels were slightly higher on presentation and during the subsequent follow-up in cases with good outcome, with significant differences only at the 4-week interval (Fig. 2). DD and TFPI showed similar levels in both outcome groups, with statistical differences at 24 and 12 weeks, respectively (Figs. 3 and 4).

Depending on the proximal or distal location of the thrombi on baseline duplex scanning, a comparison was performed for each of the four plasma markers (Table III). Only DD showed significantly higher levels in cases with proximal clots, on presentation ($p < 0.05$) and 1 and 24 weeks ($p < 0.05$) after diagnosis. The rest of the markers did not show
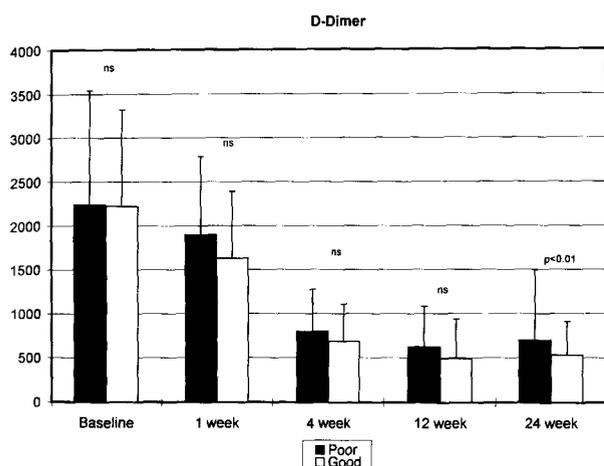
**Fig. 3.** Evolution of DD in patients presenting DVT (See explanation in Fig. 1). Values expressed as mean and SD (ng/ml). Independent *t* testing.
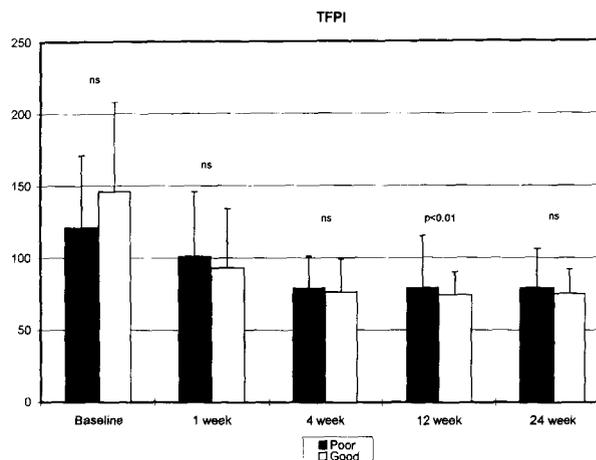


**Fig. 4.** Evolution of TFPI in patients with DVT (See explanation in Fig. 1). Values expressed as mean and SD (ng/ml). Independent *t* testing.

statistically significant differences between proximal and distal clots.

TFPI values were higher on presentation in patients receiving heparin (150 ng/ml) than in those who did not receive this drug (121 ng/ml); however, this difference was not statistically significant ($p = 0.14$). PAI levels were significantly higher in patients receiving heparin on baseline testing (15.8 ng/ml versus 14.8 ng/ml, $p < 0.05$). Heparin did not significantly influence levels of t-PA or DD. No statistically significant correlations could be found between plasma markers and age, nor among them at the different intervals studied.

## DISCUSSION

A newly developed venous thrombus induces prompt activation of the fibrinolytic system. In some cases, this plasminogen-plasmin system will completely dissolve the fresh clot early in the course of the disease. This will depend on the age and size of the clot. Besides, almost from its formation, the clot undergoes a process of organization initiated by white blood cells and endothelial cells. Most large clots cannot be completely removed by the fibrinolytic system and become organized as fibroblasts and capillaries proliferate within the thrombus. Then the clot may be incorporated into the vein wall or develop clefts as capillaries connect and form channels through which there is blood flow. This latter process is known as *recanalization.*

Our main objective was to assess the long-term time-courses of a number of specific markers of hemostasis and fibrinolysis in patients admitted with

deep vein thrombosis and treated with standard anticoagulant treatment. Another objective was to correlate those markers with long-term DVT outcome. We selected two markers, t-PA and PAI; enzymes related to the activation and inhibition of the plasminogen-plasmin system, respectively. On the other hand, DD is a fibrin degradation product being increasingly used for the diagnosis of DVT because the levels of DD are elevated in the presence of a thrombus. We decided to incorporate TFPI, a recently described serine protease synthesized by endothelial cells, into the panel of markers. TFPI is currently considered the factor Xa–dependent inhibitor of the tissue factor coagulation pathway and might play a key role in the action of heparin.

Our results indicate that most patients showed improvement of the DVT process within the 6-month follow-up period. Similar results have been reported previously by us and by other investigators who also used ultrasonography.[3,7,14] In this study, DVT outcome has not been significantly influenced by factors such as proximal extension of the clot or degree of vein obstruction caused by the thrombus on presentation.

Both t-PA and PAI are derived from the endothelium. It is for that reason that a gentle blood-drawing technique, discarding the first 4 to 5 ml, is very important to prevent contamination of the sample caused by t-PA or PAI released from the vein wall at the site of injection. Because there is evidence of fluctuations in the plasma levels of these markers during the 24-hour period, all blood extractions were done in the morning to prevent this influence.

**Table II.** Evolution of the different plasma markers during 6 months (mean ± SD)

| Marker | Baseline | 1 week | 4 weeks | 12 weeks | 24 weeks |
|---|---|---|---|---|---|
| PAI (ng/ml) | 15.2 ± 14 | 13.4 ± 13 | 14.2 ± 10 | 17.9 ± 12 | 15.5 ± 14 |
| t-PA (ng/ml) | 14.3 ± 6 | 21.5 ± 8* | 13.8 ± 8 | 12.4 ± 5* | 11.5 ± 4* |
| DD (ng/ml) | 2230 ± 1229 | 1784 ± 840† | 751 ± 450* | 565 ± 450* | 620 ± 660* |
| TFPI (ng/ml) | 132 ± 56 | 98.5 ± 42* | 78 ± 22* | 77.5 ± 29* | 77.6 ± 23* |

*$p < 0.01$ paired $t$ test compared to baseline.
†$p < 0.05$ paired $t$ test compared to baseline.

**Table III.** Evolution of the different plasma markers and level of DVT (mean ± SD)

| Marker | Level | Baseline | 1 week | 4 weeks | 12 weeks | 24 weeks |
|---|---|---|---|---|---|---|
| PAI | Proximal | 14.7 ± 14 | 15.5 ± 14 | 14.2 ± 11 | 21.1 ± 12 | 17.5 ± 15 |
|  | Distal | 16.7 ± 15 | 8.4 ± 8 | 16.1 ± 8 | 14.5 ± 11 | 15.3 ± 10 |
| t-PA | Proximal | 15.3 ± 6 | 22.3 ± 8 | 14.1 ± 8 · | 13.2 ± 5 | 11.4 ± 4 |
|  | Distal | 11.1 ± 4 | 18.8 ± 8 | 12.8 ± 6 | 9.5 ± 4 | 11.7 ± 3 |
| DD | Proximal | 2241 ± 1697 | 1789 ± 697 | 803 ± 481 | 550 ± 470 | 655 ± 749 |
|  | Distal | 2190 ± 1101* | 1766 ± 1287* | 582 ± 314 | 620 ± 419 | 512 ± 324* |
| TFPI | Proximal | 134 ± 54 | 102 ± 44 | 83 ± 22 | 81 ± 31 | 82 ± 24 |
|  | Distal | 124 ± 66 | 85 ± 36 | 64 ± 20 | 64 ± 17 | 66 ± 16 |

*$p < 0.05$ independent $t$ test comparing proximal and distal.

PAI levels did not experience significant changes after DVT diagnosis during the 6-month follow-up period. There was dramatic interindividual variation in the plasma levels of PAI, especially at presentation and after 1 week, as illustrated by the very high standard deviations shown in Table II. Plasma PAI levels were significantly higher, on baseline and 1 week later, in patients with incomplete thrombus resolution. On the other hand, PAI levels were slightly higher in the good outcome group at 12 and 24 weeks, but without statistically significant differences.

A recent study has reported that ex vivo spontaneous lysibility of human thrombi was inversely related to the amount of PAI in the thrombus.[10] Besides, PAI increase has been considered recently as a predictive factor of postoperative thromboembolism.[15] On the basis of our results, it could be hypothesized that high plasma levels of PAI early after DVT diagnosis might indicate defective fibrinolysis of the newly formed clot, which will undergo a process of organization associated with incomplete resolution. However, on the basis of the number of cases included in this study, we realize that this hypothesis is preliminary and should be validated in larger samples. We cannot explain why PAI levels, on presentation, were significantly higher in patients who were receiving heparin. On the basis of the comparisons mentioned above, we cannot attribute the difference to a significantly higher rate of proximal thrombi in patients already receiving heparin. Besides, as shown in Table

III, PAI levels were similar in patients with proximal and distal thrombi throughout the study period.

Plasma levels of t-PA increased significantly during the first week after DVT diagnosis, to decline to baseline values after 1 month. Then t-PA levels were gradually reduced 3 and 6 months thereafter. This marker was unable to distinguish patients undergoing complete DVT resolution from those who did not.

DD was increased at the time of DVT diagnosis, thus confirming previous reports demonstrating the value of this marker for the diagnosis of DVT.[16,17] DD levels remained very high 1 week after DVT diagnosis and then were reduced to values slightly above normal. Similar results have been recently reported by Elias et al.[13] Our results do not show a different time-course of DD levels in patients with complete DVT resolution and in those with incomplete clot removal. However, a recent French trial showed that DD values were higher on presentation in patients with poor DVT outcome than in patients whose conditions improved or remained stationary.[18] In contrast to some previous reports,[11] we, as did others previously,[18] have found a correlation between DD levels and the extent of venous thrombosis at presentation and during follow-up.

TFPI was slightly increased at presentation and during the first week, decreasing subsequently to normal values. Such high levels of TFPI observed within the first week after DVT diagnosis could be

attributed to TFPI release induced by the heparin treatment, as previously reported.[19,20] However, we could not document significant differences in TFPI levels at presentation in patients receiving heparin and those who did not. On the other hand, there was not a correlation between TFPI levels and DVT outcome. Whereas some studies have shown decreased levels of TFPI in patients with thromboembolic disease,[21] others have reported similar plasma TFPI levels in patients with a positive phlebography result and in those with a negative phlebography result.[22]

In conclusion, this preliminary study shows that PAI levels, early in the course of DVT, could be related to thrombus outcome. The other plasma assays—t-PA, DD, and TFPI—were unable to differentiate the patients who underwent a complete thrombus resolution, as assessed by duplex ultrasonography, from those who did not. These findings provide further insight into the process of thrombosis resolution and may help to identify those patients with poor prognosis. However, we realize that these preliminary data must be validated in a larger patient population.

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