The Laboratory Diagnosis of Venous Thromboembolic Disease by Measurement of Fibrinogen/Fibrin Degradation Products and Fibrin Monomer*

Victor Gurewich, M.D.,** Michael Hume, M.D.,† and Michael Patrick, M.D.‡

The utility of two blood tests for screening patients with suspected venous thromboembolic disease was evaluated. Eighty-two patients who underwent venography and 38 patients who were investigated with radioisotope lung scans or pulmonary angiograms were included. At the time of their radiographic study, their blood was tested for fibrinogen/fibrin degradation products and fibrin monomer by the staphylococcal clumping test (SCT) and the serial dilution protamine sulfate (SDPS) test. Patients in whom the diagnosis of pulmonary embolism or symptomatic deep vein thrombosis was confirmed had a significantly higher incidence as well as more strongly positive blood tests compared to the remaining patients. The SCT was more sensitive but the SDPS test was found to be more specific. The study indicates that these tests are sufficiently practical and precise to be used to help resolve the differential diagnosis in suspected venous thromboembolism. When both tests were negative, significant thromboembolic disease was not found. Evidence is presented suggesting that the coagulation products measured represent the result and not the cause of the thrombosis.

The clinical diagnosis of venous thromboembolic disease is notoriously difficult and imprecise. Postmortem studies have revealed that the diagnosis was made antemortem in less than 50 percent of patients with significant pulmonary embolism. The reliability of clinical diagnosis of deep vein thrombosis (DVT) is of similar magnitude, with many errors in overdiagnosis as well as underdiagnosis. Considerable interest is being given to the validation of the clinical diagnosis of venous thromboembolic disease by angiography of the legs and lungs and radioisotope scanning of the legs (125I-labelled fibrinogen) and lungs (131I-macroaggregated albumin). In addition, flow measurement by ultrasound and impedance plethysmography represent two noninvasive techniques that may improve diagnostic accuracy.

These advanced methods require time, expertise, expense and, in some instances, associated discomfort and even a small risk to the patient. None is well suited for routine clinical screening of patients for venous thromboembolism.

In the present study, two simple laboratory tests were performed in patients being evaluated for either acute DVT or pulmonary embolism. The tests surveyed were: 1) the staphylococcal clumping tests (SCT) sensitive to the degradation products of fibrinogen (FDP) and fibrin (fdp); 2) a protamine sulfate (PS) paradoxal coagulation (nonenzymatic fibrin formation) test previously shown to be...
specific for fibrin monomer and early fibrin degradation products (fdp). Only patients in whom the diagnosis was either established or excluded by angiographic and/or radioisotope methods were included. The results indicate that when both tests are negative, the presence of acute venous thromboembolic disease may be virtually excluded.

METHODS AND MATERIALS

PATIENT SELECTION

Deep Vein Thrombosis (DVT)

All patients who underwent technically successful venography were included in the study. Patients were selected for venography for two reasons: First, when the diagnosis of DVT was considered clinically because of suggestive symptoms and signs; second, patients under observation with the 125I-labelled fibrinogen leg scan who developed a positive scan after operation. This latter group was asymptomatic and classified as such. They were derived from a series of patients at the New England Baptist Hospital being routinely screened for DVT by the radioisotope leg scan following total blood sample was positive had at least one follow-up lung scan. Interpretations were all made by independent observers from the department where the study was performed.

None of the patients in the pulmonary embolism group was also in the deep vein thrombosis group.

Venography

A technique similar to that described by Lewis and Dale was used. The dorsal vein of the great toe was utilized for injection whenever possible since it provides the most direct access to the deep venous system. A continuous drip of saline solution containing heparin (200 units per liter) is maintained through a scalp vein needle. After plain films are taken, the table is elevated to 45° from the horizontal, the patient’s weight being borne by the leg not under examination. A tourniquet is applied just above the ankle of the leg under examination and a cuff is placed around the thigh of the opposite limb. After disconnecting the saline solution, contrast medium is slowly injected (45 ml) after which time they were examined with a high intensity lamp over a black, nonreflective background. The serial dilutions made the test semiquantitative. The presence of a feathery appearance (fy), fibrin strands (fs) or a gel (g) in any of the tubes constituted a positive reaction. An amorphous precipitate alone without any fibrin-like material represents a negative reaction. This is invariably found in the 1:5 and often the 1:10 dilutions and is due to precipitated fibrinogen.

The Staphylococcal Clumping Test (SCT)

The test was performed according to the method previously described. In brief, serial dilutions of PS ranging from 1:5 to 1:40 were made up with 0.05 M tromethamine (TRIS) buffered saline, pH 6.5. TRIS buffer at this pH is stable for about one month when kept refrigerated (4°C).

To 1.0 ml of platelet-poor citrated plasma, 4 drops of aprotonin (Trasyol) were added. Plasma (0.2 ml) was added to equal volumes of each of the PS dilutions. After careful mixing, the tubes were allowed to incubate overnight at room temperature (20-23°C) after which time they were examined with a high intensity lamp over a black, nonreflective background. The serial dilutions make the test semiquantitative. The presence of a feathery appearance (fy), fibrin strands (fs) or a gel (g) in any of the tubes constituted a positive reaction. An amorphous precipitate alone without any fibrin-like material represents a negative reaction. This is invariably found in the 1:5 and often the 1:10 dilutions and is due to precipitated fibrinogen.

LABORATORY STUDIES

All blood tests were performed by the same laboratory personnel, none of whom were involved in the clinical aspect of the study and who had no knowledge of the clinical findings. In all cases, the blood sample was collected within 48 hours prior to the diagnostic venogram or lung scan. The sample was kept on ice during the period between collection and laboratory processing whenever possible. The importance of a clean venipuncture and careful mixing of blood collected for plasma was emphasized to the personnel responsible for the blood collections. Blood for serum was treated with thrombin (5 NIH units per ml) to ensure complete removal of clottable protein, and with epsilon-aminocaproic acid (10 mg/ml) to prevent in vitro proteolysis.

The Serial Dilution Prostaminate Sulfate (SDPS) Test

PS paracoagulation was performed by the SDPS test method as previously described. In brief, serial dilutions of PS ranging from 1:5 to 1:40 were made up with 0.05 M tromethamine (TRIS) buffered saline, pH 6.5. TRIS buffer at this pH is stable for about one month when kept refrigerated (4°C).

To 1.0 ml of platelet-poor citrated plasma, 4 drops of aprotonin (Trasyol) were added. Plasma (0.2 ml) was added to equal volumes of each of the PS dilutions. After careful mixing, the tubes were allowed to incubate overnight at room temperature (20-23°C) after which time they were examined with a high intensity lamp over a black, nonreflective background. The serial dilutions make the test semiquantitative. The presence of a feathery appearance (fy), fibrin strands (fs) or a gel (g) in any of the tubes constituted a positive reaction. An amorphous precipitate alone without any fibrin-like material represents a negative reaction. This is invariably found in the 1:5 and often the 1:10 dilutions and is due to precipitated fibrinogen.

exposure including the upper thigh and iliac veins is taken. The patient is returned to the horizontal position, the saline-heparin solution reconnected and the leg elevated and massaged in order to empty the venous system of all contrast material. The saline-heparin solution is kept open and running for at least ten minutes before the needle is withdrawn.

CHEST, VOL. 64, NO. 5, NOVEMBER, 1973
RESULTS

Deep Vein Thrombosis (DVT)

A total of 82 patients were studied. In 46, the venogram was positive and indicative of deep vein thrombosis. Among this group, 29 were symptomatic and 17 were asymptomatic. In another 36 patients with clinical symptoms or signs suggestive of DVT, the venogram was normal. The extent of thrombosis involved more proximal veins in those patients with symptoms. The asymptomatic group consisted of patients in the 3rd-21st day following total hip replacement surgery whose DVT was detected by the radioactive fibrinogen leg scan and subsequently confirmed by venography (Table 1).

In the group with a positive venogram and clinical symptoms, the SDPS test was positive in 25 (86 percent) patients, with the positive tests ranging from 1:5 fs to 1:40 g, but in most instances giving at least a 1:20 fs reaction. The SCT titer was significantly elevated (≥ 1:8) in 24 (92 percent) patients, ranging from 1:8 to 1:128. SCT titers of <1:8 are considered within the normal range and were listed as negative (Neg). In none of the patients in the symptomatic group were both tests negative (Table 2). In the group of patients who had a positive venogram but were asymptomatic, the SDPS test was positive in only four (24 percent) patients, with the positive tests ranging from 1:10 g to 1:20 fs. The SCT titer was elevated in 14 (82 percent) patients, ranging from 1:8 to 1:32 (Table 1).

In the 36 patients in whom the venogram was negative, clinical follow-up supported the radiographic findings since most of the patients responded to symptomatic therapy alone and none of them developed pulmonary emboli despite the fact that they were not given anticoagulant therapy. The SDPS test was positive in only four (11 percent) patients, with the positive tests ranging from 1:10 g to 1:40 fs. The SCT titer was elevated in ten (40 percent) patients, ranging from 1:8 to 1:32. In only one of the patients with a normal venogram were both tests positive (Table 1, 2).

The mean SCT titer, corresponding to the µg/ml of FDP/βdpl, was calculated by giving a "negative" (<1:8) the numerical designation 2. The mean values were 9, 16 and 29 for the negative, asymptomatic and symptomatic DVT groups, respectively. The difference between the negative and symptomatic DVT groups was significant (p<0.01) (Fig 1).

Pulmonary Embolism

In 21 patients, the clinical diagnosis of pulmonary embolism was confirmed by characteristic findings on the initial and follow-up lung scans. In each instance, the diagnosis of pulmonary embolism was reported independently by a radiologist as the most likely explanation for the perfusion defect in relation to the plain film findings. In eight of the patients, the diagnosis was further validated by angiography. Of 21 patients, 16 had a positive SDPS test ranging from 1:5 fs to 1:40 g, with most patients having at least a 1:20 fs reaction. In 13 of 14 patients, an elevated SCT titer was found. In none of the patients were both tests negative (Table 3, 4).

In 17 patients, the diagnosis of pulmonary embo-
NO ASYMPT. NO PULM. DVT DVT DVT PULM. EMBOL. EMBOL.

FIC; UHE 1. SCT titer or FDP/fdp concentration found at time of venography or lung scan in five groups. Difference between no deep vein thrombosis (DVT) and symptomatic DVT is significant (p <0.01). Difference between no pulmonary embolism and pulmonary embolism is significant (p <0.001). Laboratory results

The mean concentration of FDP/fdp was 8 and 60 μg/ml for the negative and positive groups, respectively. The differences in the titers in the two groups was significant (p<0.001) (Fig 1).

**DISCUSSION**

A number of laboratory tests have been used in the diagnosis of pulmonary embolism including the determination of serum enzymes, bilirubin and arterial oxygen tension (Po2). In a careful study by Sasahara et al10 of 72 patients with pulmonary embolism, only 64 percent showed the characteristic pattern of an elevated lactic dehydrogenase (LDH) level and normal serum glutamic oxaloacetic transaminase value (SGOT), and only 18 percent showed the triad reported by Wacker et al11 of an elevated LDH and bilirubin level with a normal SGOT value. Measurement of the arterial Po2 breathing room air is a more sensitive screening test. Szucs et al12 reported an arterial Po2 of less than 80 mm Hg in each of 36 patients studied. However, Miller and Sutton13 found a normal arterial oxygen saturation in 4 of 23 patients with massive pulmonary embolism studied within 48 hours of their clinical episode. The test has the disadvantage of requiring an arterial puncture which may bleed if treatment with heparin or more particularly with a thrombolytic drug is instituted.

Several laboratory methods are available for the detection of certain soluble derivatives of fibrinogen, which are indicative of thrombin or plasmin action. The SCT is sensitive to the fibrinolytic products of fibrinogen and fibrin. It is sensitive to all FDP/fdp in serum either directly, or indirectly as a result of soluble complex formation with fibrin monomer during the process of blood clotting.14 The SCT gives results comparable to those obtained by immunologic methods performed on serum.14,15 The PS-induced paracoagulation reaction by the SDPS method appears to be specific for fibrin monomer and early fdp7 and sensitive to concentrations of 1.0 mg percent or more.10 These products may also be detected by elution chromatography as demonstrated by Fletcher et al.16 However, this method is too laborious and technically difficult to be readily

**Table 3—Summary of Findings in 38 Patients with Suspected or Established Pulmonary Embolism.***

<table>
<thead>
<tr>
<th></th>
<th>Confirmed</th>
<th>Unconfirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No.</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Age, Yrs. Mean:</td>
<td>54</td>
<td>52</td>
</tr>
<tr>
<td>Range:</td>
<td>(20-87)</td>
<td>(22-83)</td>
</tr>
<tr>
<td>Underlying condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postoperative:</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Estrogens:</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>Cong. hrt. failure:</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Pneumonia:</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Coronary dis.:</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Miscellaneous:</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Laboratory results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDPS (+):</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Mean titer:</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>SCT (+):</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Mean titer:</td>
<td>60</td>
<td>8</td>
</tr>
</tbody>
</table>

*Results of laboratory findings expressed as in Table 1.

---

**Table 4—Difference Between SDPS (+), Both Tests (+) and Both Tests (−) in Two Groups is Significant (χ-Square p Value <0.01).**

<table>
<thead>
<tr>
<th>Laboratory Results</th>
<th>Both SDPS (+)</th>
<th>SCT (+)</th>
<th>Both Tests (+)</th>
<th>Both Tests (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Scan Positive</td>
<td>16/21</td>
<td>14/15</td>
<td>9/15</td>
<td>0/15</td>
</tr>
<tr>
<td>Lung Scan Negative</td>
<td>1/17</td>
<td>6/16</td>
<td>0/16</td>
<td>9/16</td>
</tr>
</tbody>
</table>
available for clinical purposes. Ethanol gelation may also be used to detect fibrin monomer \textsuperscript{17} but not fdp. \textsuperscript{18} However, this method is sensitive to fibrinogen itself in high concentration \textsuperscript{18} as well as to a low molecular weight protein unrelated to thrombin or thrombin action. \textsuperscript{18} Ethanol gelation therefore lacks specificity.

The two tests used in this study are sufficiently simple to be practical for clinical purposes. The SCT can be performed and read within 45 minutes. The SDPS test will give a positive reaction within 30 minutes if the plasma concentration of fibrin monomer or fdp is 5 mg percent or higher. \textsuperscript{7} However, if negative after 30 minutes, it is kept overnight at room temperature and re-examined in the morning. The test results reported here are the overnight readings only, although the more strongly positive reactions (1:20 g or 1:40 fs and g) commonly found in acute pulmonary embolism or iliofemoral deep vein thrombosis were positive within 30 minutes of mixing the PS with the plasma.

In patients with deep vein thrombosis, in whom the diagnosis was made by the \textsuperscript{125I}-fibrinogen scan, elevated levels of FDP/fpd have been reported by Ruckley et al.\textsuperscript{20} and Wood et al.\textsuperscript{21} Significantly higher peak concentrations of serum FDP/fpd were found in patients with a positive \textsuperscript{125I}-fibrinogen leg scan after operation as compared with those with negative leg scans.\textsuperscript{21} However, a higher incidence of systemic complications could not be excluded as a cause for the difference in FDP/fpd concentrations. In the present study, only the patients with symptomatic deep vein thrombosis had significantly higher concentrations of FDP/fpd at the time of venography compared to the negative group (Fig 1). The asymptomatic positive group whose diagnosis was made by the \textsuperscript{125I}-fibrinogen leg scan and confirmed by venography had more peripheral, smaller thrombi (Table 1) and therefore presumably less substrate for the formation FDP/fpd. In all of these patients, the thrombi were usually confined to veins below the knee, considered by some investigators to rarely give rise to embolism.\textsuperscript{22}

The failure of the SCT and SDPS test to detect early thrombosis identified by the radioactive leg scan suggests that the coagulation reaction products detected by these tests represent the result and not the cause of the thrombosis. These findings are consistent with our experience in another group of more than 100 patients given \textsuperscript{125I}-fibrinogen in whom the blood tests were found to be of limited value in identifying those with positive leg scan after operation.\textsuperscript{23} However, in patients with large vessel thrombosis occurring above the knee in whom the risk of significant embolization is greatest, one or the other of the tests was invariably positive (Table 1, 2). Since the tests, in particular the SDPS test, were generally negative in symptomatic patients who had no thrombosis (Table 1, 2), the tests should help reduce the incidence of false-positive diagnoses.

In patients with pulmonary embolism, a determination of FDP/fpd appears to be particularly useful. Elevated levels have been reported by Ruckley et al.,\textsuperscript{20} Wilson et al.,\textsuperscript{24} Somnabend et al.,\textsuperscript{25} Cash et al.\textsuperscript{28} and Rickman et al.\textsuperscript{27} When this combined experience is added to our own, a total of 79 patients with angiographically or radioisotopically established pulmonary embolism is obtained, of which 72 had significantly elevated levels of FDP/fpd. The incidence of positive tests by the SCT method in patients in whom the diagnosis of pulmonary embolism was suspected but not confirmed was 3 of 19 patients reported by Rickman et al.\textsuperscript{27} and 6 of 16 in our own series (Table 3, 4) giving a combined incidence of 21 percent false-positive results by the SCT method. In addition to the incidence of positive tests, the concentration of FDP/fpd in the patients with pulmonary embolism was significantly higher than in the group in whom this diagnosis was not confirmed (Fig 1).

There was only one false-positive SDPS test in the suspected pulmonary embolism patients, but the incidence of false-negative tests was slightly higher than for the SCT. In none of the patients with proved pulmonary embolism were both tests negative (Table 4).

It is well established that many cases of venous thromboembolism go unrecognized and untreated. The present study of 120 documented cases emphasizes that overdiagnosis may also be common since in a relatively high proportion of patients suspected clinically of DVT or pulmonary embolism, the diagnosis could not be substantiated (Table 1, 3). In a recent study of 2,107 autopsies reported by Modan et al.\textsuperscript{28} the incidence of false-positive clinical diagnoses of pulmonary embolism was 61.9 percent. Statistically, overtreatment appears to have become as much of a problem as undertreatment in thromboembolic disease. The established effectiveness as well as the attendant risks of anticoagulant treatment requires that it be administered promptly but selectively.

The purpose of laboratory screening is to help exclude patients who do not have active thromboembolism and to identify a population in whom more definitive as well as more laborious and costly diagnostic procedures are indicated. The measurement of FDP/fpd by the SCT appears to be a sensitive laboratory method for the detection of significant venous thromboembolic disease. The SDPS test is
somewhat less sensitive but is more specific, giving fewer false-positive results. A similar clinical experience with the SDPS test was recently published by Schorn who found that each of 18 patients with proved pulmonary embolism had strongly positive tests, whereas only a small incidence of weakly positive tests was found in patients with a variety of other diseases. Both the SCT and the SDPS test appear to reflect the presence of active thrombosis and thrombolysis rather than a state of incipient thrombosis ("hypercoagulability"). When both of the tests are negative, acute pulmonary embolism or significant deep vein thrombosis may be virtually excluded (Tables 2, 4).

ACKNOWLEDGMENT: We would like to acknowledge with gratitude the valuable contributions of T. X. Kuriakose, M.D., Linda Zuch, R.N., and Elisabeth Hutchinson, B.S.

REFERENCES
17 Godal HC, Abildgaard U: Gelation of soluble fibrin in plasma by ethanol. Scan J Haematol 3:342-351, 1966
23 Hume M, Gurewich V: Unpublished observations