Evidence for Abnormalities in Clotting and Thrombolysis as a Risk Factor for Stroke

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Abstract:
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Four hundred six patients with ischemic thrombotic cerebrovascular disease (ITCVD) and 115 age-matched controls were studied to select risk factors which would identify ITCVD-prone individuals from a healthy population. The following factors were evaluated: soluble fibrin, plasminogen, plasminogen activator, fibrinogen, partial thromboplastin time, generation of thromboplastin, fibrin degradation products, triglycerides, type IV hyperlipoproteinemia, and cholesterol. Discriminate function analyses were used to select those risk factors which best separate and classify the ITCVD and control subjects. The primary risk factors are the activated partial thromboplastin time, soluble fibrin, fibrinogen, and plasminogen activator. Utilizing only these four primary risk factors in a discriminate function, 93.2% of the patients were correctly classified. Consideration of other variables increased still further the discriminate function.

Additional Key Words: soluble fibrin, fibrinogen, plasminogen activator, thromboplastin

The plasma content of fibrinogen is elevated and fails to return to normal levels in patients who have recovered from coronary occlusion and who are not on anticoagulant therapy.1-6 This abnormality is correlated with an increase in the rate of turnover of fibrinogen,7 fatty acid control of that turnover,8 an increased generation of thromboplastin,9,10 an increased rate of utilization of platelets,11 changes in the activity of plasma antithrombin,11,12 a deficiency in the activity of plasma antithromboplastin,13,14 an increased activity of factor VIII15. All of these changes indicate the occurrence of a general coagulation disturbance in the patient with thrombosis. In addition to evidence indicating a disturbance in coagulation factors in patients with ischemic heart disease, there is indication of a concomitant defect in the plasma fibrinolytic system.16

which system is presumably involved in limiting the extent of fibrin deposition.

Published data on patients with ischemic thrombotic cerebrovascular disease (ITCVD) are generally lacking with regard to the status of the clotting and fibrinolytic systems. An objective of analysis of clotting and fibrinolytic variables is to develop an effective tool for discriminating between the patient at high risk of ITCVD and the low risk matched control. Risk factors were selected from ten biochemical variables studied in a retrospective and modified prospective epidemiological study of ITCVD. Discriminate function analyses were employed to select those risk factors which would best detect the occurrence of ITCVD. This communication reports the results of this study.

Methods
PLASMA SAMPLES
Normal plasma samples were obtained from 115 subjects without known disease. Plasma samples were obtained from 406 subjects with ITCVD diagnosed by clinical and neurological examination, lumbar puncture, electroencephalography, and cerebral blood flow studies. Biochemical evaluations were made on an average of six months following the last acute episode. The criteria for selection excluded amyotrophic lateral sclerosis, ischemic heart disease, and the absence of medications known to influence the metabolism of clotting and fibrinolytic factors.

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643
ASSAY OF PLASMA FIBRINOGEN

Blood specimens were assayed for fibrinogen according to a method used previously in this laboratory.9,7

RATE OF TURNOVER OF FIBRINOGEN

The rate of turnover of fibrinogen was measured by the C14 method developed in this laboratory.7

ASSAY OF SOLUBLE FIBRIN (F8)

Preparation of Plasma

Glycine ethyl ester-C14 (9.8 mc/mmole) and p-tosyl-l-arginine methyl ester HCl (TAMe) were obtained commercially. Blood for measurement of circulating soluble fibrin was drawn by venipuncture into a plastic syringe. Nine parts of blood were added to a polystyrene centrifuge tube containing one part of an anticoagulant mixture consisting of 2% EDTA and 10 mg per milliliter of soybean trypsin inhibitor (SBTI). The samples were centrifuged at 6,000 rpm in a Sorvall refrigerated centrifuge (Model 2B) for ten minutes at 0°C and the supernatant platelet-poor plasma was recovered and stored at 0°C. Assays were performed immediately after the samples were obtained.

Factor XIII Preparation, Assay, and Activation

Factor XIII (fraction 5) was isolated as described by Loewy7 and its activity estimated according to their method. This fraction was used as enzyme for the incorporation of glycine ethyl ester-C14. Factor XIII was activated by incubating the crude factor in cysteine, calcium and thrombin* for 15 minutes at 37°C. Thrombin activity was quenched at the end of the activation period by adding di-isopropyl-fluorophosphate (DFP), 5 x 10-4 M. Activated factor XIII, 21 μl/ml, was used immediately in the reaction mixture for the labeling of soluble fibrin.

Labeling Fibrin

The 8% ethanol fraction of plasma was formed during a one-hour incubation period at 0°C by slowly adding 0.2 ml 40% ethanol to 0.02 M TRIS buffer, pH 7.4, to 0.8 ml of whole plasma according to the procedure of Kisker and Rush.18 The precipitate was dissolved in 0.8 ml of TRIS buffer 0.02 M, pH 7.4; 1 mg/ml SBTI; and 0.025 M TAMe, pH 7.4. TAMe, as a thrombin quenching agent, replaces hirudin which was employed in the Kisker and Rush procedure. After addition of glycine ethyl ester-C14, active factor XIII, and 0.25 M TRIS, 0.05 M NaCl buffer, pH 8.2, to the fibrinogen fraction, the mixture was incubated for 30 minutes at room temperature after which 0.1 ml of 0.25 M EDTA made up in distilled water was added to stop the action of factor XIII. The fibrinogen fraction again was precipitated from this mixture in 8% ethanol.

Recovery of Clottable Protein

The labeled 8% ethanol fraction was dissolved in 0.8 ml of 0.04 M TRIS, 0.15 M NaCl, pH 7.4, buffer containing 1 mg per milliliter SBTI, 0.2 EDTA. The fibrinogen in the fraction was clotted by adding 0.1 ml of bovine thrombin (500 NIH u/ml) in 0.04 M TRIS, 0.15 M NaCl, 0.25 M EDTA, pH 7.4, to the fraction and the clottable protein recovered after one hour of incubation with the thrombin at 37°C by removing the clot on a glass stirring loop and drying it on filter paper. After dissolving the fibrin at 37°C overnight in 3 ml of 2% monochloroacetic acid, the protein was reprecipitated with 3 ml of 14% trichloroacetic acid for one hour, washed four times with 7% trichloroacetic acid to remove any unbound radioactivity and redissolved in 3 ml of 2% monochloroacetic acid, and allowed to incubate at 37°C overnight. The protein soluble in the 2% monochloroacetic acid solution was centrifuged at 2,200 rpm for three minutes in a Clay-Adams centrifuge. The supernatant was separated from the precipitate portion. Two milliliters of 1 M NaOH were added to dissolve the precipitate, followed by the addition of 2 ml of 14% trichloroacetic acid. The mixture was allowed to stand for one hour at room temperature and then centrifuged, and the supernatant was decanted and discarded. The precipitate was washed once in distilled water and dissolved in 2 ml of 5 M NH4OH. One milliliter of the protein soluble in the 2% monochloroacetic acid and 1 ml of the precipitate dissolved in 5 M NH4OH were added to separate, pre-weighed concentric stainless steel planchets, and evaporated to dryness under a heat lamp. The planchets were then counted on a Nuclear-Chicago 183B Model Scaler with a D-47 Gas Flow Counter for ten minutes. The planchets were then re-weighed and the difference between the two weights was divided into the corrected counts per minute to establish the specific activity (SA) in counts per minute per milligram of fibrin. Total counts for F8 represent the sum of clottable protein, soluble and insoluble, in 2% monochloroacetic acid.

Specificity

Omission of factor XIII failed to produce significant uptake of glycine-1-C14 ethyl ester into the fibrin monomer.

Addition of exogenous thrombin and Ca2+ to the plasma of the test system induced up to a 30-fold increase in the yield of F8, whereas addition of exogenous fibrinogen to plasma in the absence of active thrombin did not increase the yield of F8.

PLASMINOGEN

The caseinolytic assay of plasminogen was performed as previously reported from this laboratory.19

DILUTE BLOOD CLOT LYSIS

The assay of thrombolysis is based upon the procedure of Fearnley,20 modified to permit quantitation by: (a) release of hemoglobin, (b) loss of fibrin, and (c) release of fibrin degradation products. In addition, analysis was made of clot retraction.

FIBRIN DEGRADATION PRODUCTS

Fibrin degradation products were determined according to the modification by Mertens et al.,21 of the method by Mersky et al.22

GENERATION OF THROMBOPLASTIN

Generation of thromboplastin was measured according to a modification of the method of Biggs and Douglas used in our laboratory.11 Partial thromboplastin time was according to the method of Proctor and Rappaport.23

*Donated by Parke, Davis and Company, Detroit, Michigan.
RISK FACTOR FOR STROKE

**TABLE 1**

*Increase in the Concentration of Plasma Fibrinogen in ITCVD With Reference to Age-Matched Normal Subjects*

<table>
<thead>
<tr>
<th>Age group</th>
<th>Normal</th>
<th>Patient</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–29</td>
<td>2.52 ± 0.93</td>
<td>3.41 ± 0.27</td>
<td>35.3 (P &lt; 0.001)</td>
</tr>
<tr>
<td>30–39</td>
<td>2.66 ± 0.16</td>
<td>3.66 ± 0.37</td>
<td>37.6 (P &lt; 0.001)</td>
</tr>
<tr>
<td>40–49</td>
<td>3.18 ± 0.14</td>
<td>4.08 ± 0.18</td>
<td>28.3 (P &lt; 0.001)</td>
</tr>
<tr>
<td>50–59</td>
<td>3.15 ± 0.11</td>
<td>3.76 ± 0.16</td>
<td>19.4 (P &lt; 0.001)</td>
</tr>
<tr>
<td>60–69</td>
<td>3.73 ± 0.67</td>
<td>4.09 ± 0.18</td>
<td>9.7 (P = NS)</td>
</tr>
<tr>
<td>70+</td>
<td>3.72 ± 0.08</td>
<td>3.72 ± 0.08</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>116</td>
<td>-</td>
</tr>
</tbody>
</table>

Plasma fibrinogen values expressed as milligrams per milliliters ± SE. No. = number of subjects.

TRIGLYCERIDES, CHOLESTEROL AND BETA-LIPOPROTEINS

Triglycerides were measured according to the method of Kessler and Lederer. Cholesterol determination was by the method of Technicon. Lipoprotein phenotyping was based on criteria of the World Health Organization.

**Results**

**ANALYSIS OF THE CONCENTRATION OF PLASMA FIBRINOGEN AS A FUNCTION OF ITCVD**

Table 1 summarizes the data with respect to the concentration of plasma fibrinogen in 422 subjects with and without ITCVD. The concentration of plasma fibrinogen increases in specific association with the occurrence of ITCVD (table 1 and fig. 1). The increase is statistically independent of the increase associated with aging. Groups of patients and normal subjects matched for age show that the patients exhibit a 35.3%, 37.6%, 28.3%, 19.4% and 9.7% increase, respectively, above the normal subjects for the 20 to 29, 30 to 39, 40 to 49, 50 to 59, and 60 to 69 year age groups. All of these
changes are significant excepting the last age group where only three subjects comprise the clinically normal group. From these data it is noted that the difference in the concentration of fibrinogen between the normal subject and the patient decreases as the population ages. No sex difference was detected. Evaluation of the concentration of fibrinogen as a function of age in the patient shows that the mean level of fibrinogen is not materially or consistently changed regardless of the age group, with the exception of age group 20 to 29. The mean concentration of fibrinogen in the patient, regardless of age, was 54% higher than the concentration in the normal subject in the 20 to 29 year age group and 31% higher than the mean of all subjects without clinically overt evidence of ITCVD. The concentration of plasma fibrinogen increases as the normal population ages. By age 60 to 69, the clinically normal subjects have attained a mean level of plasma fibrinogen which is 48% greater than the concentration found during age 20 to 29 years. These observations indicate a metabolic relationship between aging and those biochemical changes which relate specifically to the occurrence of ITCVD.

Rate of Turnover of Fibrinogen-C\textsuperscript{14} in ITCVD

Our previous studies have shown that the elevated plasma content of fibrinogen in coronary thrombosis is due to an enhanced rate of biosynthesis.\textsuperscript{7} Our study of the ITCVD patient suggests the same cause for the elevated levels of fibrinogen (fig. 2). The plot of log-specific activity of fibrinogen-C\textsuperscript{14} measured as fibrin-C\textsuperscript{14} versus days after injection of the precursor amino acid shows that the rate of turnover of fibrinogen in this patient was 111% greater than that observed in the concurrently run control subject, or 81% greater than that previously observed in 11 age-matched normal subjects.

Partial Thromboplastin Time (PTT) in ITCVD

The activated partial thromboplastin time in 233 patients was accelerated from an average of 42.5 seconds in 61 normals to 35.5 seconds (table 2 and fig. 3). The magnitude of this shortened time is 20% (P < 0.001); however, it is estimated that a seven-second shortening of the clotting time in the range of 45 to 30 seconds represents about a 40% increase in concentration in procoagulant factors. Of the patients, 89.4% had a PTT of 39 seconds or less.

Generation of Thromboplastin in ITCVD

Figure 4 summarizes the data with respect to the generation of plasma thromboplastin in 94 subjects.

### Table 2

**Partial Thromboplastin Time (PTT) in ITCVD Patients**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Patient Clotting time, seconds</th>
<th>Normal Clotting time, seconds</th>
<th>P value</th>
<th>Number of patients</th>
<th>Number of normals</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>35.5</td>
<td>42.5</td>
<td>&lt;0.001</td>
<td>233</td>
<td>61</td>
</tr>
<tr>
<td>20-29</td>
<td>35.6</td>
<td>42.3</td>
<td>&lt;0.005</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>30-39</td>
<td>36.8</td>
<td>44.0</td>
<td>&lt;0.05</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>40-49</td>
<td>35.6</td>
<td>41.2</td>
<td>&lt;0.001</td>
<td>27</td>
<td>19</td>
</tr>
<tr>
<td>50-59</td>
<td>34.9</td>
<td>42.3</td>
<td>&lt;0.001</td>
<td>51</td>
<td>24</td>
</tr>
<tr>
<td>60-69*</td>
<td>35.2</td>
<td>42.4</td>
<td>&lt;0.025</td>
<td>67</td>
<td>3</td>
</tr>
<tr>
<td>70- over*</td>
<td>34.8</td>
<td></td>
<td>&lt;0.001</td>
<td>70</td>
<td>0</td>
</tr>
</tbody>
</table>

*Compared with normals 50 to 59 years old.
It is evident that generation of thromboplastin is related to ITCVD regardless of the chronological age of the subject and that the rate of generation also increases with aging. The hematocrit did not show significant changes. The appearance of an age-related function in the generation of thromboplastin and the absence thereof in the partial thromboplastin time suggest that the age-related component is related to those factors which are activated by kaolin in the partial thromboplastin time. The increased rate of generation of thromboplastin is correlated with previous observations in this laboratory of a decrease in plasma content of antithromboplastin in patients with coronary thrombosis.

Assay of Soluble Fibrin Monomer (F₈) in ITCVD
Fifty-eight patients showed a 520% mean increase (P < 0.001) above 43 normal subjects in the plasma content of soluble fibrin monomer (F₈) (table 3). An increase in F₈ was found in all age-matched groups ranging from 137% (P < 0.004) in the 80 to 89 year age group, as compared to the normal 50 to 59 year age group, to a 707% increase in the 20 to 29 year age group. A partial correlation is noted between PTT and F₈ to a plasma content of 800 cpm per minute (fig. 5). This correlation may signify that the generation of thromboplastin reflects a dynamic concurrent production of thrombin and concurrent conversion of fibrinogen to fibrin. Although there is an approximate correlation between increasing plasma levels of fibrinogen and F₈, this correlation does not provide for a strict proportionality between the two variables. Thus, a low or high plasma level of fibrinogen may be found in the presence of a high content of F₈.

Plasma Plasminogen Content in ITCVD
The plasma content of plasminogen was determined by the caseinolytic assay in 134 subjects. The precursor of the fibrinolytic enzyme is elevated in the patient until the seventh decade of life, at which time a deficiency of the proenzyme appeared (table 4). Since the content of plasminogen progressively increases with age in the normal population, the difference between normals and patients diminishes as age increases until the seventh decade when there appears a reversal of the trend with the patient group exhibiting a deficiency of 11.3%.

Thrombolysis in ITCVD
Study of 86 normal subjects and 336 patients of various age groups shows that the patients display a significant deficiency in the rate of thrombolysis...
Age Range | Mean Age Yrs. | 20-30 | 30-40 | 40-50 | 50-60 | 60-70 | 20-29 | 30-39 | 40-49 | 50-59 | 60-69 | 70-79 | 80-89 |
---|---|---|---|---|---|---|---|---|---|---|---|---|---|
N | 24.3 | 26 | 4 | 12 | 6 | 10 | 58 | 3 | 6 | 10 |
A | 36.0 | 4 | 7 | 8 | 6 | 10 | 3 | 1 | 3 | 10 |
A | 38.0 | 5 | 4 | 7 | 8 | 6 | 3 | 1 | 3 | 10 |
A | 44.3 | 17 | 5 | 8 | 6 | 10 | 3 | 1 | 3 | 10 |
A | 45.1 | 6 | 6 | 8 | 6 | 10 | 3 | 1 | 3 | 10 |
A | 55.5 | 10 | 6 | 8 | 6 | 10 | 3 | 1 | 3 | 10 |
A | 54.6 | 6 | 6 | 8 | 6 | 10 | 3 | 1 | 3 | 10 |
A | 63.5 | 4 | 6 | 8 | 6 | 10 | 3 | 1 | 3 | 10 |
A | 63.6 | 4 | 6 | 8 | 6 | 10 | 3 | 1 | 3 | 10 |

**Figure 4**

Generation of plasma thromboplastin in 94 normal subjects and patients with ITCVD. Numbers at the top of each bar indicate the number of subjects in that group. Thromboplastin generation is measured in thrombin units per 0.3 ml of incubation mixture × 100 at the incubation time of maximum generation. N = normal, A = ITCVD.

A reading of 7 represents complete clot lysis, whereas a reading of 2 denotes as little as 30% release of hemoglobin (fig. 7) or only a 20% decrease in the fibrin content of the clot. Each age group of patients exhibit a deficiency in thrombolysis when compared to age-matched subjects with no overt clinical evidence of cerebrovascular disease. The 336 patients, as a group, exhibit a 26.8% deficiency relative to the 20 to 29 year age group. The magnitude and severity of the deficiency in many patients is not reflected in these data where large numbers of patients are averaged. In frequent

**Table 3**

Radioassay of Soluble Fibrin Monomer in ITCVD

<table>
<thead>
<tr>
<th>Age group</th>
<th>cpm/mg fibrin</th>
<th>% Increase</th>
<th>P value</th>
<th>Number of patients</th>
<th>Number of normals</th>
</tr>
</thead>
<tbody>
<tr>
<td>No age group</td>
<td>589</td>
<td>95</td>
<td>520</td>
<td>&lt;0.001</td>
<td>58</td>
</tr>
<tr>
<td>20-39</td>
<td>444</td>
<td>55</td>
<td>707</td>
<td>&lt;0.005</td>
<td>4</td>
</tr>
<tr>
<td>40-49</td>
<td>618</td>
<td>131</td>
<td>372</td>
<td>&lt;0.001</td>
<td>10</td>
</tr>
<tr>
<td>50-59</td>
<td>782</td>
<td>135</td>
<td>479</td>
<td>&lt;0.001</td>
<td>6</td>
</tr>
<tr>
<td>60-69</td>
<td>488</td>
<td>83</td>
<td>261</td>
<td>&lt;0.001</td>
<td>24</td>
</tr>
<tr>
<td>70-79</td>
<td>888</td>
<td>83</td>
<td>558</td>
<td>&lt;0.003</td>
<td>11</td>
</tr>
<tr>
<td>80-89</td>
<td>320</td>
<td>137</td>
<td>137</td>
<td>&lt;0.004</td>
<td>3</td>
</tr>
</tbody>
</table>

*Since adequate numbers of older clinically normal subjects were not available for the purpose of calculating the degree of significance, the patient group is compared with the 50 to 59-year-old normal group with no overt clinical evidence of cerebrovascular thrombosis.

cpm = radioactive counts per minute.
Correlation between a shortened partial thromboplastin time and the plasma content of soluble fibrin. DPM = radioactive counts per minute. o = normal, • = ITCVD.

TABLE 4
Comparison of the Plasma Levels of Plasminogen in Normal and Patient Groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Normal</th>
<th>Pathological</th>
<th>(P-M)</th>
<th>% change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30–39</td>
<td>4.43 ± 0.66*</td>
<td>5.35 ± 0.93</td>
<td>+0.92</td>
<td>+20.8</td>
<td>0.035</td>
</tr>
<tr>
<td>40–49</td>
<td>5.20 ± 0.91</td>
<td>5.81 ± 0.98</td>
<td>+0.61</td>
<td>+11.7</td>
<td>0.085</td>
</tr>
<tr>
<td>50–59</td>
<td>5.58 ± 0.68</td>
<td>5.89 ± 0.56</td>
<td>+0.31</td>
<td>+5.6</td>
<td>0.084</td>
</tr>
<tr>
<td>60–69</td>
<td>6.02 ± 0.71</td>
<td>5.34 ± 0.71</td>
<td>−0.68</td>
<td>−11.3</td>
<td>0.008</td>
</tr>
</tbody>
</table>

*Units = case in units per milliliters plasma.

This finding indicates that the defect is one of activation of plasminogen. This conclusion also is suggested by the inverse relationship between plasma content of plasminogen and the rate of thrombolysis with aging. The content of plasminogen may increase because of deficient activation. Evaluation of possible differences in clot retraction failed to show significant differences between the normal and the patient population, although retraction in the normal as measured by clot size was slightly greater. This change could not be correlated with thrombolytic activity. It may be a reflection of the higher content of plasma fibrinogen in the patient.

Fibrin Degradation Products (FDP) in ITCVD

The erythrocyte hemagglutination inhibition immunoassay was run on 92 patients and 46 normal subjects of various age groups. Of the patients, 60.9% exceeded 1.2 μg of FDP per milliliter of plasma. Of the normals, 19.6% exceeded this value (fig. 8). The apparently anomalous presence of an increase in the content of FDP occurring concurrently with a depressed activation of plasminogen may be explained by the increase in the level of the fibrin substrate available for lysis by the enzyme and possibly by defective metabolism of the FDP.

Triglycerides, Cholesterol, and Type IV Lipoproteins in ITCVD

Study of 141 patients (fig. 9) showed that only 24.8% had triglycerides in excess of 160 mg %. This value represents the National Heart and Lung Institute (NHLI) cutoff for normal subjects in the 40 to 49 year age group. The percent of patients who exceeded 140 mg %, the upper 5% NHLI cutoff for 20 to 29-year-old normal subjects, was 52.5. Of the total patients only 13.5% displayed a type IV hyperlipoproteinemia.

A similar low correlation between ITCVD and the plasma content of cholesterol was found (fig. 10). Of 141 patients studied, 35.5%, 17.7%, and 4.2% exceeded, respectively, 210, 240, and 265 mg %. These values of cholesterol concentration represent the upper 10% NHLI cutoffs for normal subjects in the 20 to 29, 30 to 39, and 40 to 49 year age groups, respectively.
TABLE 5

<table>
<thead>
<tr>
<th>Age group</th>
<th>Normal No.</th>
<th>Patient No.</th>
<th>% deficiency</th>
<th>Test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>5.34*</td>
<td>4.86</td>
<td>9.9</td>
<td>P = 0.18</td>
</tr>
<tr>
<td>30-39</td>
<td>5.10</td>
<td>4.15</td>
<td>22.9</td>
<td>P = 0.09</td>
</tr>
<tr>
<td>40-49</td>
<td>4.28</td>
<td>3.57</td>
<td>19.9</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>50-59</td>
<td>4.37</td>
<td>3.69</td>
<td>15.7</td>
<td>P = 0.03</td>
</tr>
<tr>
<td>60-69</td>
<td>4.00</td>
<td>3.75</td>
<td>6.7</td>
<td>P = 0.29</td>
</tr>
<tr>
<td>70+</td>
<td>4.22</td>
<td>99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>336</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Units of release of hemoglobin in grading system of 0 to 7.

EVALUATION OF THE DISCRIMINATORY FUNCTION
OF THE VARIOUS ASSAYS

Of the 58 patients subjected to the F8 assay, 87.9% exceeded the coefficient giving the best separation of the patients from the normal group. Of 317 patients subjected to the fibrinogen assay, 92.4% exceeded the discriminatory coefficient. In a patient group of 233 subjects, 89.4% of the population displayed a PTT smaller than the coefficient giving the best separation of the patients from the normal subjects. In thrombolysis, 309 patients out of a population of 406 patients, or 76.1%, exhibited values of lytic activity which were less than the value which gave the best separation of the two study groups. On the basis of these results, it appears that 8% to 24% of the patients may be misclassified depending on the assay. However, fibrin formation, deposition, and organization may result from defects at a number of reaction steps, e.g., enhanced formation of thrombin or depressed fibrinolysis. Hence, use should be made of a multifactorial discriminate function which takes all of the specific assays or variables into account. Calculation of the F value in a stepwise regression
RISK FACTOR FOR STROKE

FIGURE 7

Correlation between the dilute blood clot lysis (thrombolysis) color readings and the release of hemoglobin.

analysis showed that PTT, F₈, and fibrinogen account for most of the discrimination (table 6). The computer printout of the first and second canonical variables of the stepwise discriminate analysis (BMDO7M—UCLA Health Sciences Computing Facility, Los Angeles, California, 1965) are given in figure 11. The stepwise discriminate analysis as represented in this printout correctly classified 93.2% of the patients and 81% of the normals. This differential between the discriminating index for the normals versus the patients may indicate the existence or development of covert ischemic thrombotic disease. A four-variable linear discriminate function based upon PTT, F₈, fibrinogen, and thrombolysis was then determined on all subjects. The computer program for the linear discriminate function analysis and the calculation of the index, Y, designated as BMDO4M is available at the Baylor Institute of Computer Sciences, Houston, Texas. Of the patients, 93.2% also were correctly classified by this technique. Three patients were misclassified as normal subjects. However, one had a shortened PTT, one had an elevated F₈, and two of the three were borderline in thrombolysis. Of the normal subjects, 88.1% were correctly classified by the linear analysis. Five normal subjects were misclassified. Three displayed a shortening of the PTT and elevated fibrinogen and two showed elevated F₈.

Discussion

It is known that the elevation in the plasma content of fibrinogen in the patient with coronary thrombosis is not of a transient nature and therefore is to be distinguished from the short-lived increase found in stress or tissue injury and the consequent repair process, e.g., burns, trauma, infection, abscess, etc. The cause of this elevation in the plasma content of fibrinogen is increased biosynthesis and an enhanced rate of turnover. The metabolic pathway for the enhanced turnover of fibrinogen cannot be determined from turnover data. However, there is much indirect evidence to suggest that the pathway for the enhanced turnover in thrombosis is via the fibrin route. For example, there is a correlation between the enhanced turnover of fibrinogen and (a) an increased generation of
thromboplastin,1,9-11 (b) a deficiency in the activity of plasma antithromboplastin,11 (c) an increased rate of platelet utilization,12 and (d) an increase in factor VIII.15 Coupled to these observations are those data which indicate a disturbance in the fibrinolytic system.16

The problem of determining the pathway taken by the enhanced turnover of fibrinogen in thrombosis and the question of whether the enhanced generation of thromboplastin is potential or dynamic is probably academic in concept since both are closely associated clinically with the formation and retention of fibrin and thrombosis. Nonetheless, the answering of these questions is crucial. The problem in bringing this knowledge to bear at the clinical level has not been so much the lack of significant correlations between changes in the clotting and fibrinolytic factors and thrombosis as it has been a lack of clear understanding of the meaning of the data and, hence, its significance and potential application. This lack of understanding has indeed led to dissent as to whether the coagulation disturbances can be equated with thrombosis even though the disturbance is most highly correlated with thrombosis. For example, low levels of coagulation factors occurring in patients with congestive cardiac failure, a condition commonly associated with thrombosis,27 have been cited as evidence against a causal relationship between hypercoagulability and thrombosis. A low level of coagulation factors in the presence of intravascular clotting is probably due to an enhanced utilization of the clotting factor, a rate of utilization which exceeds the biosynthetic replacement capacity. Evidence for this interpretation may be found in several studies. In the "defibrination syndrome" or acute disseminated intravascular coagulation, a precipitous decline in the content of plasma fibrinogen is associated with thrombosis.28 An increased rate of turnover of fibrinogen accompanied by elevated plasma concentrations is the usual metabolic picture encountered in the thrombosis patient and is indicative of pathophysiological steady state kinetics.7 The issue of whether oral anticoagulants improve the mortality statistics is controversial. It has been suggested that conventional oral anticoagulant therapy does not impede the formation of arterial thrombi. This has led to seeking explanations alternative to hypercoagulability with the result that arterial thrombosis has been attributed to a morphological entity, namely, the platelet. However, platelet aggregation has not been shown to be stable in the absence of fibrin,29 thus once again indicating an essential role for hypercoagulability and formation of fibrin. Where the level of anticoagulation therapy has been rigorously controlled, a significant drop in recurring thrombosis and mortality has been observed.30-32 There are other clinical observations which equate hypercoagulability with fibrin formation and deposition. Arteriosclerosis obliterans is associated with chronic hypercoagulability although thrombosis per se is not a frequent observation.33

PILGERAM, CHEE, VON DEM BUSSCHE

TABLE 6
Stepwise Discriminate Analysis (BMDO7M)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal</th>
<th>Patient</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTT</td>
<td>42.31 ± 3.81</td>
<td>34.95 ± 6.71</td>
<td>38.62*</td>
</tr>
<tr>
<td>Soluble fibrin</td>
<td>122.76 ± 81.24</td>
<td>630.89 ± 626.95</td>
<td>26.59*</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>3.01 ± 0.61</td>
<td>4.04 ± 1.01</td>
<td>11.23*</td>
</tr>
<tr>
<td>DBCL</td>
<td>4.35 ± 0.89</td>
<td>3.67 ± 1.76</td>
<td>0.010 &gt; F level</td>
</tr>
</tbody>
</table>

*Significant at 1% level.
Idiopathic recurrent thrombophlebitis accompanied by chronic hypercoagulability is often found in younger patients without evidence of vascular disease at the site of the new thrombus. These observations have led Owen to state that it seems as logical to attribute thrombosis to hypercoagulability as to blame hemorrhage on hypocoagulability. Penick noted that the large number of conditions in which there seems to be enhanced cellular aggregation or accelerated fibrin formation, occurring in association with clinical thrombosis, makes it difficult to ignore hypercoagulability as a significant factor in thromboembolic disease.

The pathway taken by fibrinogen in its enhanced rate of turnover must be identified to determine whether hypercoagulability can be equated with intravascular coagulation and thrombosis. In this study it is shown that a patient with cerebral vascular thrombosis has an increase of 520% (P < 0.001) in the generation of circulating soluble fibrin monomer above normal. This increase of circulating soluble fibrin monomer suggests that at least part of the pathway is by way of intravascular formation of fibrin. This increased production of fibrin suggests that the increase in thromboplastin generation or the shortened partial thromboplastin time represents a dynamic active production of thrombin and fibrin rather than an unrealized potential. These observations not only indicate the physiological intravascular formation of fibrin in young normal subjects, but also show that hypercoagulability is equated with increased intravascular formation of fibrin.

Whether the enhanced formation of fibrin results in the generation of potentially occlusive intravascular deposits of fibrin-platelet thrombi must depend upon the balance between the rate of formation of fibrin and the rate of metabolism or lysis of the fibrin monomer or polymer. That the fibrinolytic activity of the patient with thrombosis is deficient, and in some cases severely so, is demonstrated by the increase in the plasma content.
of plasminogen and the deficient rate of thrombolysis. The increase in the concentration of plasminogen could be interpreted as indicative of enhanced turnover as was found to be the case with increased levels of fibrinogen. This does not appear to be the case as indicated by our finding a deficiency in activation, by an increase in the concentration of plasminogen in thrombosis, and by reports that EACA, an inhibitor of the activation of plasminogen, will induce an increase in the plasma concentration of plasminogen. Also, diffuse capillary thrombosis has been reported in a patient treated with EACA. This latter observation is in support of our findings which indicate that a pathway for the metabolism of fibrinogen is via fibrin. Fibrin must have formed if inhibition of fibrinolysis results in thrombosis. If the fibrinolytic pathway becomes impaired, or overwhelmed by overproduction of fibrin, a consequence appears to be intravascular deposition of fibrin plus platelets at anatomical sites which provide physical impedence, e.g., arterial bifurcation, or chemical alteration, e.g., epinephrine-induced endothelial injury or surgical trauma. The deficiency in thrombolysis in the patients with ITCVD indicates a deficiency in activation of plasminogen or an excess of anti-activator. A deficiency in activator is consistent with the increase in the plasma content of plasminogen and with our finding that the defect in thrombolysis is not evident in the presence of an exogenous activator of plasminogen, e.g., streptokinase.

Whether or not the very high discriminate function of the computerized multivariate linear analysis is a useful index for detecting patients who are at high risk of ITCVD depends ultimately on the answer to the question of whether the clotting and fibrinolytic changes have a causal relationship to thrombosis or whether they are secondary to the thrombotic event. Data are available which bear upon this question. First and foremost are the metabolic and biochemical data which show that the clotting and thrombolytic changes found in stroke are equated with highly significant increases in circulating soluble fibrin monomer or intravascular clotting. It is impossible for an increase in fibrin monomer to have occurred in the absence of these changes because these biochemical changes are essential to the formation of fibrin. Even the stability...
of the platelet thrombus has been shown to depend upon fibrin. For a thrombotic stroke to have occurred, there also must have occurred an increased formation and retention of fibrin. This interrelated sequence of reactions indicates that these clotting and thrombolytic changes must have occurred before the stroke in order for the precipitating cause of the stroke, namely, the thrombus, to have formed.

Secondly, the large numbers of subjects evaluated in this study provide a modified prospective study. In the traditional prospective study one follows a statistically significant number of subjects over a sufficiently long period of time and determines whether specific changes precede the occurrence of a thrombotic episode in the patient. There is available another approach to this problem and it circumvents the expense and difficulty in successfully pursuing a long-term mass screening study as well as obviating the serious time lag in securing meaningful data. In a conventional prospective study one measures the changes with aging or time which precede the ictus in a given subject. However, changes which precede the ictus also may be detected where no specific subject is followed as a function of time but where a sufficient number of subjects of various age groups are evaluated, so that the mean changes reflect that which will develop in the average subject in a conventional prospective study. The studies reported here on fibrinogen, thrombolysis, plasminogen, and generation of thromboplastin qualify in this respect. Taking fibrinogen as an example, one can demonstrate changes between each age group not only as
the population ages but also with regard to the occurrence of ITCVD. One observes an increment with age and an additional increment with the occurrence of ITCVD which is superimposed upon the aging increment. Regression analyses of these data show a progressive merging of the normal population into a patient population. Thus, we observe biochemically and graphically that which we experience clinically, namely, that as the population ages there is an increasing incidence of ITCVD and an increasing incidence of change in the clotting and thrombolytic factors. If the changes were a consequence of ITCVD, one should not observe the progressive change occurring with time in a normal population. On the basis of the foregoing considerations it seems reasonable to tentatively conclude that the clotting and fibrinolytic changes precede ITCVD and that the high degree of discriminatory function inherent in the multivariate linear analysis may provide a useful tool for detecting patients at high risk of ITCVD.

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RISK FACTOR FOR STROKE


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