A Study of Blood Coagulation Following an Acute Stroke

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Abstract:
A battery of blood coagulation tests were performed on 14 acute stroke patients. Various abnormalities were found, notably elevation of blood fibrinogen, factor VIII, prothrombin, platelet factor 3, and a shortened P.T.T. The significance of these findings is discussed and they are considered to be of no importance since similar changes were noted in the blood of healthy subjects of the same age range.

ADDITIONAL KEY WORDS: hypercoagulable state, thromboembolic cerebrovascular disease, platelet

Introduction

Although atherosclerosis of extracranial and intracranial arteries has the principal etiological role in thrombotic cerebrovascular disease, it is probable that fluctuations in blood flow and blood coagulability are also important and may determine the onset of thrombosis in the chronically diseased artery. Despite increasing knowledge of thrombus formation and clot fibrinolysis the importance of these in relation to the pathogenesis of thrombotic occlusion of cranial arteries is unclear.

In a search for a possible "hypercoagulable state" and to elucidate the role of blood coagulation mechanisms, studies have been performed in patients with thromboembolic cerebrovascular disease. Increased levels of various coagulation factors have been found in some stroke patients; however, similar increases have been found in normal individuals while other patients with definite thrombosis have normal values. An additional problem of such studies is the difficulty of relating changes in the circulating level of coagulation factors to the liability of in vivo thrombus formation.

This study reports the results of the analysis of various coagulation factors in the blood of 14 stroke patients during the acute period of their illness. The results are compared with values obtained from healthy individuals of similar age.

Methods

Coagulation studies were performed on 14 patients shortly after their admission to the hospital. Nine patients were first studied within 24 hours, ten within 48 hours, 11 within 72 hours, and the remainder after 72 hours. An attempt was made to study the patients daily until their clinical course stabilized. Nine patients were studied four times, three were studied three times and two were studied five times. All patients were in the age range of 54 to 85 and were diagnosed as having acute onset completed strokes due to thromboembolic cerebrovascular disease involving the cerebral hemispheres. No patient was diagnosed as having transient ischemic attacks, stroke-in-evolution or cerebral hemorrhage. The patients were fully investigated having brain scan, lumbar puncture, and serial electroencephalograms. Further confirmation of diagnoses was obtained in some patients with cerebral angiography.

Brain thromboplastin and cephalin were prepared from human brain according to the methods of Hjort and Bell and Alton, respectively. Diluting fluid II (DFII) was prepared from human plasma.
according to Waaler. Russell's viper venom was used as Stypven (Burroughs-Wellcome).

Blood from patients and normal controls was drawn by the two-syringe technique. Nine volumes of blood were mixed with one volume of 3.13% sodium citrate dihydrate and centrifuged at 2000 × g (4°C) for 30 minutes for platelet-poor plasma separation. Platelet-rich plasma was obtained by centrifuging blood at 200 × g for 10 minutes at 4°C or room temperature (the latter for platelet-aggregation studies). Glass contact was avoided by the use of plastic syringes, tubes, and pipettes.

Normal control plasma and platelets were obtained from adult male laboratory workers (age range 20 to 30) whose level of coagulation factors was determined by comparison with 15 to 30 other normal males as previously described. In some instances, a lyophilized standard plasma (Fibro-Trol, Citrated) was used, but only after its coagulation factors were determined by comparison of several vials of each lot with fresh normal calibrated human plasma.

Platelets were counted by an electronic particle counter (Coulter) as described by Harker and Finch. The Quick one-stage prothrombin time (PT) was performed on intact plasma according to Biggs and Macfarlane except that calcium chloride was used at 0.02 M concentration.

The coagulation screening tests and factor assays were performed by published methods as follows: kaolin-partial thromboplastin time (kaolin-PTT); thrombin time (TT); urea clot solubility; fibrinogen; factor II (prothrombin); factor V (proaccelerin); factor X (Stuart); and fibrinogen degradation products (FDP). Serum specimens for FDP determination were collected according to Israels et al. and adsorbed twice with one-half volume of fresh sheep erythrocytes before being tested. Factor VIII was measured in a kaolin-PTT system using hemophilia A plasma as substrate.

Platelet factor 3 release was estimated by adding patient platelet-rich plasma to normal platelet-poor plasma to a final platelet concentration of 50,000 per cu mm. A kaolin-recalcification time was performed in duplicate on the mixture.

Clot retraction was measured by adding patient platelet-rich plasma to normal platelet-poor plasma to a final platelet concentration of 100,000 per cu mm. Then 0.5 ml of the mixture was recalcified with 0.1 ml of 0.05 M CaCl₂ in a 10 × 75 mm Pyrex tube (previously flamed to a yellow color for ten seconds and then cooled to room temperature). Thirty minutes following clot formation (37°C), clot retraction was measured from the interior tube bottom to the lower edge of the clot and expressed in millimeters.

Platelet aggregation was measured in a Chrono-log Aggregometer by the method of Born and Cross using the following reagents and final concentrations: adenosine diphosphate (ADP), 1 × 10⁻⁶M; bovine thrombin (UpJohn), 0.2 NIH units per milliliter; epinephrine, 1 × 10⁻⁶M; collagen (Sigma Chemical Co.), a 2% suspension in 0.15 M saline was centrifuged ten minutes at 200 × g (4°C) and the supernatant diluted tenfold in the platelet-rich plasma being studied.

Platelet adhesion to glass was studied as follows: native blood was passed without delay from a disposable plastic syringe through Tygon tubing (B-44-4x) using a Harvard Peristaltic Pump (Model 1201, set at position No. 1, giving a flow rate of 10.3 ml per minute) to a plastic tubing (125 mm in length) containing 1 gm of glass beads (Superbrite type 070, 3M Co.). Tubing and beads were as described by Salzman and were used as supplied by the manufacturer. The stock container of beads was mixed before each sample was removed to prevent size stratification by settling. Effluent blood from Salzman tube (10 ml per minute) was collected in five 10 × 75 mm Falcon plastic tubes (F2054) containing 0.02 ml of 5% disodium EDTA to a final volume of 1.0 ml. After the fifth tube was collected, the glass bead tube was disconnected, and a control tube was collected by pumping blood from the syringe through B-44-4x Tygon tubing to the collection tube. This control tube was compared to a second control prepared by passing blood directly from syringe to collection tube, thus bypassing the pump. The platelet counts of the two control tubes did not vary significantly. Platelet counts in the five fractional collection tubes were compared with the control tubes (averaged), and the platelet adhesion was calculated by difference. A population of 25 normal individuals were tested by this method, and the results, expressed as percent adhesion ± S.D., for the five fractions were: fraction I, 19 ± 12%; fraction II, 43 ± 18%; fraction III, 61 ± 17%; fraction IV, 73 ± 15%; fraction V, 81 ± 11%. This method is similar to that described by Bowie et al., differing mainly in the use of native rather than heparinized blood, a smaller column of beads, a faster flow rate, and a peristaltic perfusion pump.

To assess the effect of age on certain coagulation tests, these were performed on a population of 26 individuals (age 55 to 90) who had only trivial complaints or desired routine physical examinations. Some were receiving drugs, but none of these were known to influence coagulation tests. A kaolin-PTT and assays for fibrinogen, prothrombin, factor VIII (AHF) and platelet factor 3 were performed on plasma samples from these individuals.
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Results
Each patient had from three to five coagulation studies in the acute stage of their stroke. The results are given in table 1.

- **PT**—Nine of the 14 patients had all prothrombin times within the normal ranges. Four of the remaining five had at least one normal PT.

- **Kaolin-PTT**—All 14 patients tended to have a short PTT.

- **TT**—Eight patients had all tests in normal range. The remaining six had slight increases in TT, but with some normal values also.

- **Urea Test**—Was negative in all 14 cases, i.e., the count was insoluble in urea.

- **Platelet**—Count was normal in 12 patients, slightly low in two.

- **Plt factor 3**—Was normal in six and increased in eight, three of whom had no PF 3 assay in the normal range.

- **Clot retraction**—Was normal in 11 patients, slightly decreased in three patients, two of whom had at least one normal test.

- **Glass adhesion**—Was normal in all 14 patients.

- **Platelet aggregation**—Was normal in 11 out of 12 in whom the test was performed.

- **Factor assays**—Fibrinogen level was normal in only one patient; it was low in one and elevated in 12, only two of whom had at least one normal level.

- **Factor II**—Was normal in four, increased in nine with four of these patients having at least one normal level.

- **Factor V**—Was normal in seven, decreased in one, increased in five. All patients had at least one normal level.

- **Factor VIII**—Was normal in six, slightly elevated in eight patients, seven of whom had at least one normal level.

- **Factor X**—Was normal in ten patients, slightly low in one, slightly increased in two.

- **Fibrinogen degradation products**—Were normal in 13 patients, and increased in one patient on two occasions.

The results of estimations of fibrinogen, factor II, factor VIII, PTT and platelet factor 3 performed on healthy subjects who were presented for routine physical examination or had trivial complaints and who were in the age range of our stroke subjects are presented in table 2. It should be noted that a significant proportion of the values were beyond the usually accepted range of normal as were the values of the stroke patients.

Discussion
Only limited information is presently available regarding abnormalities of blood coagulation in the acute thromboembolic stroke. In studies previously reported only minor aberration of questionable significance have been noted. A recent collaborative Japanese-American study by Ettinger et al. reported the findings using a battery of coagulation tests performed on the blood of patients who had sustained an acute stroke. The patients were studied within seven days of the ictus and tests were repeated eight to 30 days later and again after 30 days. Their study of PT, PTT, platelet adhesiveness and fibrinogen level revealed a shortened PTT during the acute stroke period in both Japanese and American patients which was sustained into the recovery period in the American patients. They demonstrated no increase of platelet adhesiveness but found a definite increase of blood fibrinogen with maximal increase occurring in the later stages. The relationship of these findings to the ones presently reported and their possible significance will be discussed.

Our own finding of a significant elevation of plasma fibrinogen in 12 of 14 patients confirms the results of Ettinger et al. This rise of plasma fibrinogen in the patient with acute stroke has been previously observed and has been considered a nonspecific reaction to injury since similar increases of blood fibrinogen is seen following burns and surgery, and has been found in association with infection, rheumatoid arthritis, multiple sclerosis and cancer. Elevated fibrinogen levels have also been reported in a significant number of healthy elderly people, and our study confirms this. It would, therefore, appear that this finding is of little significance.

We have demonstrated, as did Ettinger et al. a shortened PTT, but since similar values were noted in our control subjects of the same age little value can be placed on this finding.

Our study of factors II, V, VIII, and X failed to reveal any significant abnormality from our control group although some patients and controls had minor deviations from the accepted normal values. These factors have not previously been studied in the acute stroke patient.
TABLE 1

Results of Coagulation Tests in 14 Patients with Acute Strokes

<table>
<thead>
<tr>
<th>Test screen</th>
<th>Normal</th>
<th>M.E. 57 M</th>
<th>L.W. 73 F</th>
<th>J.C. 52 M</th>
<th>G.B. 85 F</th>
<th>M.F. 63 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT, sec.</td>
<td>14-16</td>
<td>15.5-18.1</td>
<td>N</td>
<td>14.8-17.3</td>
<td>11.9-13.7</td>
<td>N</td>
</tr>
<tr>
<td>Kaolin-PTT, sec.</td>
<td>35-53</td>
<td>27.1-33.4</td>
<td>31-36.6</td>
<td>28.7-31.4</td>
<td>28.29</td>
<td>29.5-33.7</td>
</tr>
<tr>
<td>TT, sec.</td>
<td>12-19</td>
<td>17.1-23.1</td>
<td>14.3-21.7</td>
<td>17.1-21.3</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Urea test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Count, per mm³ thousands</td>
<td>150-400</td>
<td>109-146</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Platelet F. 3, sec.</td>
<td>45-60</td>
<td></td>
<td>40.4-52.9</td>
<td>41-51</td>
<td>N</td>
<td>38-44</td>
</tr>
<tr>
<td>Clot retraction, mm</td>
<td>3-7</td>
<td>N</td>
<td>2-4.5</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Glass adhesion</td>
<td>73 ± 15</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>% 4th &amp; 5th fraction</td>
<td>81 ± 11</td>
<td></td>
<td></td>
<td></td>
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<td>Aggregation</td>
<td>Normal</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
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<tr>
<td>Factor assays</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fibrinogen, mg%</td>
<td>200-400</td>
<td>611-735</td>
<td>299-537</td>
<td>508-567</td>
<td>123-158</td>
<td>N</td>
</tr>
<tr>
<td>Factor II, % normal</td>
<td>75-125</td>
<td>93-132</td>
<td>132-144</td>
<td>144-152</td>
<td>N</td>
<td>120-140</td>
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<tr>
<td>Factor V, % normal</td>
<td>75-140</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<tr>
<td>Factor VIII, % normal</td>
<td>50-200</td>
<td>189-267</td>
<td>171-298</td>
<td>184-259</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Factor X, % normal</td>
<td>75-125</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Fibrinogen degradation</td>
<td>Products, μg/ml</td>
<td>&lt;6</td>
<td>N</td>
<td>19.5</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Number in parentheses above patient's initials indicates number of coagulation studies. Number and letter below patient's initials indicates age and sex. Letter N indicate studies were normal for a particular test.

Furthermore, an extensive study of blood coagulation factors in normal subjects by Brakman et al. demonstrated a wide variation of individual factors, emphasizing the limited importance of small and variable fluctuations in coagulation factor values. We have, therefore, concluded that the minor elevations of factors VIII and II found in some patients is of no significance.

Before concluding that a “hypercoagulable” state does not exist in patients in whom a thromboembolic stroke develops, the capability of this type of study to reveal such a state should be considered. It is quite conceivable that the analysis of inactive clotting factors would not reveal a state of increased blood coagulability.

The difference between analysis of inactive factor concentration and factor activity has been emphasized by Wessler, who showed that infusion of large amounts of inactive factor X into rabbits failed to induce thrombo-

TABLE 2

Results of Coagulation Tests in 26 Healthy Control Subjects in Age Range 55 to 90

<table>
<thead>
<tr>
<th>Test</th>
<th>Number of subjects</th>
<th>Age (range)</th>
<th>Range</th>
<th>Test value</th>
<th>Mean ± SD</th>
<th>Number of subjects beyond normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (200-400 mg%)</td>
<td>15</td>
<td>59-90</td>
<td>233-870</td>
<td>525 ± 156</td>
<td>12</td>
<td></td>
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<tr>
<td>Factor II (75-125% normal)</td>
<td>19</td>
<td>59-90</td>
<td>101-194</td>
<td>133 ± 24</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Factor VIII (50-200% normal)</td>
<td>19</td>
<td>59-90</td>
<td>147-405</td>
<td>222 ± 69</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>PTT (35-53 sec)</td>
<td>26</td>
<td>55-90</td>
<td>26.9-49.3</td>
<td>33.3-4.3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Platelet Factor 3 (45-60 sec)</td>
<td>15</td>
<td>56-83</td>
<td>39.9-54.8</td>
<td>45.9-4.6</td>
<td>9</td>
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TABLE 1  (continued)

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<tr>
<td>Men</td>
<td>55 F</td>
<td>72 F</td>
<td>54 M</td>
<td>70 M</td>
<td>65 F</td>
<td>84 M</td>
<td>60 F</td>
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<td>32.8-33.3</td>
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<td>29.7-31.7</td>
<td>26.9-32.4</td>
<td>26.1-29.6</td>
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<td>109-134</td>
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<td>525-621</td>
<td>420-636</td>
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<td>164-184</td>
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<td>123-161</td>
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<td>96-184</td>
<td>139-224</td>
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<td>N</td>
<td>N</td>
<td>206-450</td>
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<td>177-288</td>
<td>146-258</td>
<td>180-250</td>
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sis, while small amounts of activated factor X resulted in massive thrombi in jugular veins.

It has been suggested\textsuperscript{32, 33} that the elevated fibrinogen level might adversely affect cerebral blood flow by increasing blood viscosity and thus might predispose to thrombosis. Eisenberg,\textsuperscript{21} however, showed that there is a poor correlation between fibrinogen levels and blood viscosity.

In a study of so-called secondary aggregation or “clumping” that is associated with release of ADP-like activity from platelets, Danta\textsuperscript{84} presented evidence that induction of the second phase of platelet aggregation is dependent on the ADP concentration and on the platelet count of the plasma. He also found that the smallest concentration of ADP needed to bring about second-phase clumping was less in 70 patients studied at least three months after a stroke than in control subjects. Danta emphasized the uncertainty whether platelet aggregation in vivo is similar to the aggregation reactions observed in the laboratory. Platelet counts and platelet aggregation were normal in our patients.

In the present study, a single concentration of ADP (1 × 10^{-8}M) was used and no attempt was made to detect the lowest concentration of ADP sufficient to induce secondary aggregation. Furthermore, our patients were studied shortly after the acute stroke rather than three months or more afterward. Thus Danta's data and our own are not strictly comparable.

A new method for detecting intravascular coagulation has been developed by Fletcher and Alkaerts.\textsuperscript{85} They propose that where true hypercoagulability or intravascular thrombosis exists, a proportion of plasma fibrinogen molecules will be found in a complexed high molecular weight form. Their method revealed a "hypercoagulable" state in some of our patients who had essentially normal blood coagulation by conventional methods. These studies will be published in detail elsewhere.

Finally, one might consider that the minor abnormalities of coagulation factors in our stroke and control patients are significant, and that our healthy control patients represent a population predisposed to stroke. We consider this highly unlikely but only follow-up investigation will furnish proof.
We conclude from our results that factor analysis is not a sensitive tool with which to search for a "hypercoagulable" state.

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