Fifteen patients with acute leukemia were found to have evidence of a generalized hemostatic disorder. These patients could be divided into three groups. The first group consisted of three patients with increased fibrinogen catabolism without clinical or laboratory evidence of intravascular coagulation. The second group of five patients had laboratory evidence of intravascular coagulation without clinically evident bleeding or thrombosis. The third group of seven patients developed symptomatic intravascular coagulation characterized by bleeding, renal failure, and poor response to platelet transfusions. Laboratory evidence for intravascular coagulation in these patients included falling plasma fibrinogen and factor V levels and elevated serum levels of fibrinogen degradation products. Heparin therapy resulted in clinical improvement in all seven patients. Rising plasma fibrinogen and factor V levels correlated with a beneficial clinical response to heparin. Increased fibrinogen catabolism, asymptomatic intravascular coagulation, and symptomatic intravascular coagulation form part of a spectrum of generalized hemostatic disorders in acute leukemia.

HEMORRHAGE, a frequent complication of acute leukemia, is usually attributed to thrombocytopenia. The following observations have provided evidence for a generalized coagulation disorder in leukemia: (1) Coagulation factors have occasionally been decreased in leukemia.1-3 (2) Increased fibrinolytic activity has been reported in sporadic cases of acute leukemia, and the use of fibrinolytic inhibitors has rarely resulted in a beneficial effect.4 (3) Accelerated intravascular coagulation (AIC) has been reported most commonly in acute promyelocytic leukemia,5-8 although a few patients with other forms of leukemia have been described.9-12

In this report we have studied seven patients with various forms of acute leukemia (AL) who developed AIC. Their clinical background, hemorrhagic complications, coagulation abnormalities, and response to heparin are presented. Investigation of an additional 16 patients with acute leukemia revealed that 30% had coagulation abnormalities indicative of AIC. 125I-fibrinogen survivals were performed in three additional patients with acute leukemia. Shortened half-lives and increased fractional catabolic rates were found in all three patients. Hence, increased fibrinogen catabolism, subclinical AIC, and symptomatic AIC form a spectrum of coagulation abnormalities in acute leukemia.

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MATERIALS AND METHODS

Blood from patients was collected in nonwettable syringes in 1/100 volume of 40% sodium citrate or 1/50 volume of 20% potassium oxalate. Plasma was separated after centrifugation at 3000 rpm at 4°C for 15 min (1600 g). All coagulation studies were performed immediately, or the sample was frozen at −30°C and tested within 48 hr.

Coagulation Studies

Coagulation and fibrinolytic studies were performed by the following techniques: platelet count by the method of Bull et al.,13 the prothrombin time by the method of Quick,14 utilizing rabbit brain thromboplastin (Ortho Diagnostics, Raritan, N.J.); and the partial thromboplastin time by the method of Langdell et al.,15 using Celite as the activator (General Diagnostics, Morris Plains, N.J.). The thrombin time was performed by the method of Pedan and McFarland.16 The Reptilase time was measured in a similar manner. Reptilase, venom of Bothrops atrox, was obtained in powdered form as a gift from Pentapharm Ltd., Basel, Switzerland. It was dissolved in distilled water and used at a concentration of 20 μg of venom/ml. Factor V was assayed by the method of Stefanini;17 factor VII-X by the method of Owren and Aas;18 and factors VIII and IX by a modification of the method of Simone et al.19 Fibrinogen was determined by two methods: tyrosine determination on the fibrin clot,20 and by the method of Clauss.21 The clotting time was performed by a modified method of Lee and White.22

The euglobulin clot lysis was performed by the Milstone method.23 The plasminogen assay was modified from the method of Alkjaersig et al.;24 α-casein (Worthington Biochemical, Freehold, N.J.) was used as the substrate, and peptides not precipitated by perchloric acid were measured at 275 nm. Fibrinogen degradation products were measured by tanned sheep red cell hemagglutination inhibition25 and by immunodiffusion.26

Preparation of Fibrinogen

Blood from a normal individual was collected in 4% sodium citrate, and the plasma was separated by centrifugation at 3000 rpm for 15 min. The plasma was then recentrifuged at 3000 rpm for 15 min, and the cell-free plasma was harvested. The plasma was diluted with an equal volume of 0.09 M sodium citrate and centrifuged again at 3000 rpm at 4°C for 15 min. Saturated (NH₄)₂SO₄ was added with stirring to the plasma-citrate solution until 25% saturation at room temperature was achieved. The mixture was stirred for an additional 10 min after all the (NH₄)₂SO₄ had been added and was then allowed to stand at room temperature for an hour. The mixture was centrifuged for 10 min at 4°C at 2000 rpm. The precipitate was suspended in 0.09 M (NH₄)₂SO₄, centrifuged at 1200 rpm for 10 min, and the precipitate was redissolved in 0.005 M sodium citrate (usually between 5% and 10% of the original plasma volume). An aliquot of the fibrinogen was tested for clottable protein, and the remainder was frozen at −60°C until used. (The per cent clottable protein in these fibrinogen preparations varied between 90% and 96%).

Thrombin

Bovine thrombin (Parke-Davis, Detroit, Mich.) was dissolved in 50% (v/v) glycerol in normal saline. Further dilutions were made with normal saline just prior to its use.

Clottable protein was measured by diluting purified fibrinogen 1:40 in 0.005 M sodium citrate. This solution was read at 280 nm in a spectrophotometer (reading D₅) with the 0.005 M sodium citrate as the blank. The fibrinogen solution was clotted at room temperature with 0.1 ml of bovine thrombin (100 U/ml). In addition, an equal amount of thrombin
was added to 4.0 ml in a control tube of 0.005 M sodium citrate. The clot was removed after 120 min (if necessary the tube was centrifuged). The supernatant (D2) and the thrombin citrate control solution (D3) were read at 280 nm in a spectrophotometer with 0.005 M sodium citrate as the blank. Clottability was calculated by the formula:

\[
\frac{[D_1 - (D_2 - D_3)]}{[D_1]}
\]

**Fibrinogen Survival**

Homologous fibrinogen was iodinated with \(^{125}\text{I}\) by the method of MacFarlane\(^{27}\) to a ratio of 0.5 mole iodine/mole of fibrinogen. After iodination, excess iodine was removed by precipitation of the fibrinogen with saturated (NH\(_4\))\(_2\)SO\(_4\) (final concentration, 25% saturated) and repeated washing of the precipitate with 1 M (NH\(_4\))\(_2\)SO\(_4\) in 0.005 M sodium citrate, until the activity of the last wash was less than 0.5% of the activity of the first supernate. The \(^{125}\text{I}\)-fibrinogen was redissolved in 0.005 M sodium citrate. The removal of all excess iodine was confirmed both by measuring the supernate after TCA precipitation of the iodinated fibrinogen and by thin-layer chromatography performed by the Radiopharmacy of the National Institutes of Health. The final activity was approximately 7 \(\mu\)Ci/mg fibrinogen. The iodinated fibrinogen was filtered through Millipore filters (0.45 \(\mu\)m) and was tested for sterility and pyrogenicity by the Radiopharmacy, National Institutes of Health.

The subject received saturated solution potassium iodide (SSKI) 2 days before and throughout the study. A dose of 30 \(\mu\)Ci of \(^{125}\text{I}\)-fibrinogen in 4 or 5 ml of 0.005 M sodium citrate with 1% normal serum albumin (human) as stabilizing protein was given intravenously. Samples of blood were collected 15 min, 1 hr, 2 hr, 4 hr, 8 hr, and at least once a day thereafter. Twenty-four urine collections were made over the duration of the experiment. On each sample, the plasma radioactivity, TCA precipitability of the radioactivity (2-hr and 24-hr clottabilities), and fibrinogen concentrations were determined. Identical studies were performed on 21 normal volunteers for control values. The data were analyzed by a least squares program on a Control Data 3200 computer. All plasma and fractionated fibrinogen from normal donors were tested for hepatitis-associated antigen. In addition, hepatic enzyme studies were performed on the donor plasma at the time of each plasmapheresis.

**RESULTS**

Seven patients with acute leukemia, two in relapse, and five in the initial phase of their disease presented with elevated peripheral white cell counts, large numbers of circulating blast cells, bone marrow replacement, and extramedullary sites of leukemic infiltration (Table 1). Within 1–7 days after admission, the patients developed hematuria, epistaxis, ecchymoses, oozing from venipuncture and/or bone marrow aspiration sites, and, in two patients, renal failure. AIC occurred in two patients in relapse, M.M. and M.K. The white blood count rose rapidly while they were receiving chemotherapy. In the five newly diagnosed patients, the white cell count and percentage of blasts had decreased at the time of the onset of AIC (Table 1). In addition, the extramedullary sites of leukemic infiltration decreased in size or were no longer clinically detectable.

The most consistent coagulation abnormalities (Table 2) in these patients were prolonged prothrombin times, long thrombin times, low factor V levels, increased fibrin(ogen) degradation products, and thrombocytopenia. Fibrinogen levels varied from normal to low. The one-stage factor VIII level was normal or elevated in six patients. Factor VII-X was low in five patients, and
Table 1. Patient Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Disease</th>
<th>White Blood Count (× 10⁶/cu mm)</th>
<th>Blasts (%)</th>
<th>Platelets (× 10⁹)</th>
<th>Blood Urea Nitrogen</th>
<th>Other Organs Involved</th>
<th>LDH</th>
<th>Chemotherapy</th>
<th>Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.K.</td>
<td>6</td>
<td>ALL</td>
<td>44 148</td>
<td>40 27</td>
<td>104 45</td>
<td>14 7</td>
<td>Liver</td>
<td>3,300</td>
<td>POMP</td>
<td>V.P.†</td>
</tr>
<tr>
<td>M.M.</td>
<td>18</td>
<td>ALL</td>
<td>10 125</td>
<td>5 97</td>
<td>189 31</td>
<td>19 40</td>
<td>Spleen, testicles, lymph nodes</td>
<td>2,100</td>
<td>HU L-Asp</td>
<td>B.M.§</td>
</tr>
<tr>
<td>B.A.</td>
<td>15</td>
<td>AML</td>
<td>44 1.3</td>
<td>97 0</td>
<td>31 26</td>
<td>15 19</td>
<td>Liver, spleen</td>
<td>1,400</td>
<td>PRAVDA</td>
<td>V.P.†</td>
</tr>
<tr>
<td>K.McT.</td>
<td>12</td>
<td>ALL</td>
<td>28 2.2</td>
<td>74 10</td>
<td>31 3</td>
<td>18 57</td>
<td>Liver, spleen</td>
<td>3,200</td>
<td>POMP</td>
<td>B.M.</td>
</tr>
<tr>
<td>G.G.</td>
<td>17</td>
<td>LSAL</td>
<td>112 31</td>
<td>89 84</td>
<td>53 23</td>
<td>38 18</td>
<td>Liver, spleen, lymph nodes</td>
<td>12,000</td>
<td>HU Cytosine-Arabinoside POMP</td>
<td>B.M.</td>
</tr>
<tr>
<td>K.S.</td>
<td>15</td>
<td>ALL</td>
<td>154 52</td>
<td>97 65</td>
<td>95 26</td>
<td>14 10</td>
<td>Liver, spleen, lymph nodes</td>
<td>2,200</td>
<td>POMP</td>
<td>V.P.†</td>
</tr>
<tr>
<td>R.E.</td>
<td>3.5</td>
<td>ALL</td>
<td>31 6</td>
<td>31 0</td>
<td>98 13</td>
<td>22 80</td>
<td>Liver, spleen, lymph nodes</td>
<td>29,000</td>
<td>POMP</td>
<td>B.M.</td>
</tr>
</tbody>
</table>

* A, value at time of admission.
† B, values at time of AIC.
ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; LSAL, lymphosarcoma cell leukemia; LDH, lactic dehydrogenase; HU, hydroxyurea; L-asparaginase; POMP, prednisone, vincristine, methotrexate, 6-MP; PRAVDA, prednisone, vincristine, daunomycin, L-asparaginase.
†† Bleeding from venipuncture sites.
§§ Bleeding from bone marrow sites.
Table 2. Coagulation Studies at Time of AIC

<table>
<thead>
<tr>
<th>Patient</th>
<th>Prothrombin Time (sec)</th>
<th>PTT (sec)</th>
<th>Thrombin Time (sec)</th>
<th>Reptilase Time (sec)</th>
<th>ECL (min)</th>
<th>Fibrinogen</th>
<th>Factor V (%)</th>
<th>Factor VII-X (%)</th>
<th>Factor VIII (%)</th>
<th>Factor IX (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.K.</td>
<td>16.8</td>
<td>43.3</td>
<td>58</td>
<td>31</td>
<td>&gt;180</td>
<td>1.54</td>
<td>326</td>
<td>398</td>
<td>512</td>
<td>41</td>
</tr>
<tr>
<td>M.M.</td>
<td>29.2</td>
<td>43.2</td>
<td>47</td>
<td>37</td>
<td>&gt;180</td>
<td>0.48</td>
<td>67</td>
<td>22</td>
<td>64</td>
<td>9</td>
</tr>
<tr>
<td>B.A.</td>
<td>33.5</td>
<td>52.0</td>
<td>&gt;180</td>
<td>&gt;180</td>
<td>&gt;180</td>
<td>1.30</td>
<td>75</td>
<td>64</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>K.McT.</td>
<td>14.5</td>
<td>28.3</td>
<td>42</td>
<td>N.D.</td>
<td>&gt;180</td>
<td>1.88</td>
<td>157</td>
<td>N.D.</td>
<td>64</td>
<td>39</td>
</tr>
<tr>
<td>G.G.</td>
<td>17.4</td>
<td>41.9</td>
<td>48</td>
<td>26</td>
<td>&gt;180</td>
<td>1.97</td>
<td>225</td>
<td>N.D.</td>
<td>256</td>
<td>35</td>
</tr>
<tr>
<td>K.S.</td>
<td>22.7</td>
<td>31.4</td>
<td>&gt;180</td>
<td>57</td>
<td>&gt;180</td>
<td>2.19</td>
<td>159</td>
<td>56</td>
<td>256</td>
<td>41</td>
</tr>
<tr>
<td>R.E.</td>
<td>14.4</td>
<td>42.6</td>
<td>53</td>
<td>28</td>
<td>&gt;180</td>
<td>N.D.</td>
<td>221</td>
<td>380</td>
<td>128</td>
<td>56</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Changes in fibrinogen (black circles) and factor V (open circles) before and after initiation of heparin therapy in six patients with acute leukemia with AIC. Fibrinogen values are on the left ordinate and the factor V values on the right ordinate of each graph.

The factor IX level was low in one patient. The Reptilase time was prolonged in six patients; however, the degree of prolongation was not as great as was the prolongation of the thrombin time. The plasminogen level was low in five of six patients.

The lactic dehydrogenase (LDH) was markedly elevated in each patient in the absence of hepatic disease, hemolysis, or any evidence of pulmonary or cardiac disease. Schistocytes were observed in only one patient’s peripheral blood smear (G.G.).

All seven patients received intravenous heparin therapy at 50 U/kg of body weight at 6-hr intervals. In two patients, the dosage was increased before a response in the coagulation factors was evident. These two patients received 70 U/kg. The low heparin doses were used because of the inherent risk of hemorrhage in a thrombocytopenic anticoagulated patient. The clotting times were maintained at one to one and a half times baseline values.

Cessation of bleeding was noted within 24-48 hr after the initiation of heparin treatment in all seven patients. In the two patients with red cells and
hemoglobin casts in the urinary sediment, institution of heparin was followed
by increased urinary output and the disappearance of red cells and hemoglobin
from the urinary sediment.

The rise in plasminogen, fibrinogen, and factor V levels (Fig. 1) after
heparinization was prompt (within 24–48 hr) and sustained, while the changes
of other coagulation tests varied. The prothrombin time, thrombin time, and
fibrinogen degradation products returned to normal between 1 and 5 days
after heparin treatment. The factor VIII level was low in only one patient;
however, the level of factor VIII was significantly lower in four other patients
than is usually seen with acute leukemia without AIC.

In two patients the response to random donor platelets was very poor,
despite the fact that they had never previously received platelet transfusion.
After heparinization, however, platelet transfusions resulted in "normal"
posttransfusion increments. Although analysis of the effect of heparin on
platelet counts was clouded by platelet transfusions, residual leukemia, and
continuous chemotherapy, after initiation of heparin therapy the platelet count
doubled within 2 days in five patients and rose to normal levels in two of
these patients.

None of these patients had any hemorrhagic complications related to anti-
coagulation, nor was the regimen of chemotherapy altered. Four of the seven
patients went into remission after one course of chemotherapy. (Two patients
who relapsed at a later date again developed the clinical and laboratory find-
ings of AIC).

Investigation of 16 patients with AL, either in the initial pretreatment
period or in relapse with leukocyte counts of 2300–27,000, revealed that five
of these patients had elevated fibrinogen degradation products, prolonged
prothrombin, Reptilase, and thrombin times. Two patients had a low factor V
level, and one patient had a low fibrinogen level. None of these patients
developed hemorrhagic symptoms and none was anticoagulated. The abnormal
laboratory tests returned to normal within 3–10 days.

Three patients, not receiving chemotherapy, with AL (two in relapse, one
initial diagnosis) without high peripheral white counts and no evidence of AIC
received homologous 125I-fibrinogen. During the fibrinogen survival, the
plasma fibrinogen did not change. The fibrinogen half-life was shortened, and
the fractional catabolic rate was increased (Table 3).

Table 3. Fibrinogen Catabolism in Leukemia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma Volume (ml/kg)</th>
<th>Plasma Fibrinogen (mg/ml)</th>
<th>Half-Life (days)</th>
<th>Catabolic Rate (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>43.7</td>
<td>2.61</td>
<td>114.1</td>
<td>2.55</td>
</tr>
<tr>
<td>ALL</td>
<td>46.2</td>
<td>3.62</td>
<td>167.2</td>
<td>3.02</td>
</tr>
<tr>
<td>AML</td>
<td>63.0</td>
<td>3.29</td>
<td>207.5</td>
<td>1.03</td>
</tr>
<tr>
<td>Normal (21)</td>
<td>37.5</td>
<td>2.85</td>
<td>107.5</td>
<td>3.69</td>
</tr>
<tr>
<td>(± SD)</td>
<td>(9.43)</td>
<td>(0.60)</td>
<td>(34.3)</td>
<td>(0.45)</td>
</tr>
</tbody>
</table>
DISCUSSION

The incidence of AIC as a complication in acute leukemia is unknown. Individual cases of acute myelogenous leukemia,10,12 myelomonocytic leukemia9,38 and acute stem cell leukemia11 with evidence of accelerated intravascular coagulation have been reported. In reporting a large series of patients with acute leukemia, Brakman et al.29 found various coagulation abnormalities and evidence of fibrinolysis, but none of the patients was thought to have AIC. A low plasminogen level was the only abnormality that was associated with a poor clinical course. Other reports30-31 have also stressed low plasminogen levels and evidence of hyperfibrinolysis in acute leukemia. In our patients, evidence for systemic hyperfibrinolysis was lacking, and the low plasminogen values and fibrin(ogen) degradation products returned to normal after the initiation of heparin therapy. This suggests that the abnormal values were not related to decreased synthesis or primary fibrinolysis but to secondary fibrinolysis at local sites associated with AIC.

From our studies, AIC would appear to be a common, although often subclinical, complication of AL. Coagulation abnormalities related to AIC in AL can be divided into three categories. (1) Increased fibrinogen catabolism. In the three patients studied the homologous 125-I-fibrinogen survival was shortened. None of these patients was receiving any chemotherapy; they were not septic or febrile, and there was no clinical or laboratory evidence of AIC. We cannot definitely state that the increased fibrinogen catabolism was related to AIC, since fibrinogenolysis or enhanced tumor cell or normal catabolic cell(s) uptake could produce a similar result. (2) Subclinical AIC. This appears to be a clinically compensated state. Consumption occurs without evidence of a hemorrhagic diathesis. Compensation is presumably achieved by increased synthesis of coagulation factors, rapid clearance of activated coagulation factors, and a mild degree of accelerated utilization of coagulation factors. Secondary (localized) fibrinolysis prevented fibrin deposition and organ impairment. (3) Symptomatic AIC. In AL this was manifested by bleeding, oliguria with renal failure, and thrombocytopenia unresponsive to platelet transfusion in patients without previous platelet transfusions. After heparinization, the coagulation factor(s) and fibrinogen levels rose and bleeding ceased. Two patients who developed hematuria and renal failure during AIC recovered completely after heparinization. In some patients the initial level of fibrinogen and factor VIII, although "normal" initially, increased after heparinization. This suggested increased consumption from AIC that was terminated with heparin. The amount of tumor load (peripheral blood, bone marrow, and involved organs) would appear to influence the occurrence of AIC. In some patients, chemotherapy seemed to precipitate the AIC by rapidly decreasing the leukemic cell population.

Our patients with symptomatic AIC presented with marked elevations of the peripheral leukocyte count, with over 40% of the cells being comprised of leukemic blasts. In addition to a markedly hypercellular bone marrow, our patients had hepatosplenomegaly and/or lymphadenopathy. This organomegaly reflected additional areas of leukemic cell infiltration. The elevated
lactic dehydrogenase (LDH) was a useful guide to leukemic cell destruction. In the patients reported by Brakman et al., there was an excellent correlation between the five patients with major bleeding and elevated peripheral white cell counts. In the patient reported by Leavey et al., the initial white count was over 200,000. From our experience with acute leukemia and elevated leukocyte counts, it would seem reasonable to suggest that AIC played some role in these patients’ hemorrhagic diathesis.

Markedly elevated white cell counts in acute leukemia demand immediate and intensive treatment to lower the counts. Chemotherapy should be continued in all patients to control the underlying disease. This therapy may intensify or induce AIC and result in bleeding, thrombosis, or profound thrombocytopenia. In symptomatic patients with AIC, anticoagulation and platelet transfusions should be instituted, in addition to chemotherapy, to combat the high risk of hemorrhage and renal impairment.

Investigations are in progress to determine whether leukemic cells can initiate AIC by possessing procoagulant activity similar to normal polymorphonuclear leukocytes.

ACKNOWLEDGMENT

The authors wish to acknowledge the cooperation of the attending staff and clinical associates of the Leukemia Service of the National Cancer Institute in studying these patients.

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Intravascular Coagulation in Acute Leukemia: Clinical and Subclinical Abnormalities

Harvey R. Gralnick, Sally Marchesi and Harry Givelber