Liver Dysfunction Rather Than Intravascular Coagulation as the Main Cause of Low Protein C and Antithrombin III in Acute Leukemia

By F. Rodeghiero, P. M. Mannucci, S. Viganò, T. Barbui, L. Gugliotta, M. Cortellaro, and E. Dini

Protein C, a newly identified inhibitor of blood coagulation, was measured immunologically in 58 patients with untreated acute leukemias and compared with that of normal subjects. On the average, slightly lower values were found. However, the 17 patients with overt laboratory pictures of decompensated disseminated intravascular coagulation (DIC), including 11 cases with acute promyelocytic leukemia, had protein C concentrations no lower than those of the remaining 41 patients without DIC. Antithrombin III activity and antigen were normal and, like protein C, not lowered in DIC. The concentrations of both proteins were closely correlated with changes in the indexes for liver synthetic function. A subgroup of 13 patients with hyperleukocytic leukemias had lower protein C and antithrombin III, in line with the more compromised synthetic function of their livers. Our findings indicate that liver impairment rather than DIC is the main cause of the changes in the two naturally occurring inhibitors of blood coagulation.

PROTEIN C (PC) is a vitamin K-dependent plasma glycoprotein that, when activated by thrombin and the endothelial cell cofactor thrombomodulin, is a major inhibitor of blood coagulation.\(^5\) Anticoagulant properties of PC are derived from its abilities to selectively inactivate factors V and VIII\(^-\)\(^-\) and the platelet-dependent activation of prothrombin by factor Xa.\(^9\) Furthermore, PC generates a potent fibrinolytic activity in plasma.\(^10\) Congenital deficiencies of PC result in thrombotic disease.\(^11,12\) emphasizing the pathophysiologic importance of this inhibitor.

Recently, Griffin et al.\(^13\) and Mannucci and Viganò\(^14\) demonstrated that PC is very low in patients with disseminated intravascular coagulation (DIC), which is associated with a variety of diseases. To investigate further the modifications of PC in DIC and their pathophysiologic roles, we have studied PC antigen in acute leukemia, a clinical situation frequently complicated by overt DIC.\(^15\) Antithrombin III (AT III), the other major inhibitor of blood coagulation, was assayed in parallel with PC, and the relationship between the two inhibitors investigated.

MATERIALS AND METHODS

Patients

We analyzed plasma samples from 25 males and 33 females admitted consecutively to our Hematology Divisions for untreated acute leukemia. As a control group, we studied 60 healthy subjects of both sexes from the blood donors and laboratory staff. Their mean age (38.3 yr; range 23–70) did not differ significantly from that of the leukemic patients (40.8 yr; range 7–77). Plasma was prepared from citrated venous blood, snap-frozen, stored at –70°C, and tested within 1 mo.\(^16\)

Acute leukemia was diagnosed from bone marrow and peripheral blood examination, including conventional cytochemical stains. The patients were classified according to the criteria and nomenclature suggested by Bennet et al.\(^16\) Sixteen cases were classified as lymphoid leukemias, 2 of which were of type L1 and 14 type L2. Forty-two were classified as myeloid leukemias, 6 myeloblastic without maturation (M1), 12 myeloblastic with maturation (M2), 11 hypergranular promyelocytic (M3), 9 myelocytic (M4), and 4 monocytic (M5). Thirteen cases had hyperleukocytic leukemias (blast cells more than 50,000/µl): 4 were of the lymphoid type (L2) and 9 of the myeloid type (3 M1, 1 M2, 4 M4, 1 M5).

Methods

PC antigen was measured by means of the "rocket" electroimmunonassay technique, employing a monospecific antiserum against purified PC, as previously described in detail.\(^14\) The results were expressed as a percentage of a pooled normal plasma, defined as 100%. Plasma AT III activity was measured as heparin cofactor by a method based on the chromogenic substrate S-2238;\(^17\) AT III antigen was measured by electroimmunonassay as previously described.\(^18\) Results were expressed as percentages, and the reference plasma was the same as for PC.

The method for prothrombin time (human brain thromboplastin kindly supplied by Dr. L. Poller, Manchester, UK), fibrinogen (fibrin polymerization test), and serum fibrinogen-fibrin products (staphylococcal clumping test) have been previously published.\(^19\) The ethanol gelation test was that of Godal and Abildgaard.\(^20\) The synthetic function of the liver was evaluated by measuring serum albumin with a standard 12-channel autoanalyzer and serum pseudocholinesterase (PCE) by the Weber method.\(^21\)

Statistics

To obtain a normal distribution of the observed values, PC, AT III, and fibrinogen concentrations were log-transformed. Accordingly, the values are given as geometric means and 95% confidence limits (CL). The other measurements were normally distributed, and values are given as arithmetic means and 95% CL. Regression analysis was performed by the method of least squares, and the
correlation coefficient ($r$) calculated. The two-tailed Student's t tests for paired and unpaired data were employed to assess the significance of the differences between groups.

**RESULTS**

For the group of 58 patients with acute leukemias as a whole, PC antigen was significantly lower ($p < 0.001$) than for 60 controls (mean 73.8%, CL 33%-166%; versus 100.6%, 72%-139%). Twenty-three patients (39%) had levels of PC below the normal range. Mean AT III activity, assayed in parallel in only 44 patients, was not significantly (NS) lower than in controls (89.7%, 57%-143%; versus 100.4%, 70%-135%). There were, however, 9 patients (21%) who had AT III concentrations below the normal range. AT III was also assayed as AT III antigen; there were no significant differences between the results of the two assays (AT III antigen: mean 86.5%, 52%-142%; NS) and the values were positively correlated with those of the functional assay ($r = 0.67; p < 0.01$). Both functional and antigenic AT were positively correlated with antigenic PC ($r = 0.63$ and 0.71, $p < 0.01$).

Figure 1 shows the distribution of PC values in patients with acute promyelocytic leukemia (APL), acute myeloid nonpromyelocytic leukemia (AML), and acute lymphoid leukemia (ALL). Lower mean concentrations than in normal controls were found in each of these groups, with the lowest mean value in the AML group. The differences between the three groups were not statistically significant. Mean AT III activity was significantly lower than in controls only in the AML group ($p < 0.01$), whereas patients with APL and ALL had normal levels of this inhibitor (Fig. 2). AT III antigen behaved like AT III activity (data not shown).

DIC was diagnosed in 17 patients on the basis of abnormality in at least two of the following tests: fibrinogen <0.130 mg/dl, fibrinogen-fibrin degradation products >20 µg/ml, positive ethanol gelation test. No DIC was found in any patient with ALL, whereas 10 of 11 cases with APL and 7 of 31 cases with AML had DIC according to these criteria. PC and AT III were not lower in patients with DIC than in those without DIC (Table 1). The low levels of fibrinogen and the prolonged prothrombin time in the DIC group testify to the severity of the DIC in our patients (Table 1). There was no significant correlation between PC and AT III and fibrinogen concentrations in either the DIC or the non-DIC groups.

The synthetic function of the liver was evaluated

![Fig. 1. Protein C in patients with acute promyelocytic leukemia (APL), acute myeloid leukemia (AML), and acute lymphoid leukemia (ALL). The shaded area shows the normal range of protein C in healthy subjects; the bars show the geometric mean values. Comparing healthy subjects with AML, $p < 0.001$; with APL and ALL, $p < 0.01$.](image1)

![Fig. 2. Acute leukemia. Antithrombin III activity in patients with APL, AML, and ALL. Comparing healthy subjects with AML, $p < 0.01$.](image2)

| Table 1. Protein C and Antithrombin III in Patients With and Without DIC |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                             | DIC Patients               | Non-DIC Patients            | $p$                          |
|                             | ($n = 17$)                 | ($n = 41$)                  |                             |
| Protein C (%)               | 72.5                       | 73.9                       | NS                          |
|                             | (38.5-136.2)               | (30.6-174.3)               |                             |
| Antithrombin III activity (%) | 86.5                      | 89.9                      | NS                          |
|                             | (57.5-129.8)               | (54.7-147.7)               |                             |
| Fibrinogen (mg/dl)          | 101.8                      | 245.5                      | <0.001                      |
|                             | (35.1-195.5)               | (113.9-528.6)              |                             |
| Prothrombin time (sec)      | 22.3                       | 15.1                       | <0.001                      |
|                             | (17.1-29.9)                | (13.2-23.1)                |                             |

Values show means and 95% confidence limits (in parentheses).
from the laboratory indexes of albumin and PCE, which were significantly lower in leukemias than in controls (albumin: mean 3.8 g/dl, CL 2.7–5.2 versus 4.3, 3.2–5.4, p < 0.01; PCE 2,006 mU/ml, 1,627–3,909; versus 2,850, 1,900–4,000, p < 0.01). Both PC and AT III were positively correlated with these indexes (Fig. 3). There was no correlation between either PC and AT III and the number of circulating blast cells. However, patients with more than 50,000 blast cells/µl in the peripheral blood had significantly lower concentrations of both inhibitors than the patients without excess blast cells (Table 2). Hyperleukocytic patients also manifested more severe defects in the liver function tests but not in fibrinogen levels (Table 2). Indexes of DIC, such as ethanol gelation test and fibrinogen-fibrin degradation products, were the same in patients with and without hyperleukocytic leukemia (data not shown).

**DISCUSSION**

PC concentrations have not been extensively investigated in acute leukemias, but Griffin et al.\textsuperscript{1} reported low PC in two patients. The data available for AT III seem to indicate that this inhibitor is usually normal.\textsuperscript{22–26} On the whole, our study indicates that neither inhibitor is strikingly altered in acute leukemia. Mean PC concentrations were slightly lower than in normal controls in the entire group of patients with acute leukemia, with about one-third of the cases having PC values below the normal range. Mean AT III concentrations were not significantly reduced, although about one-fifth of the patients had low values. AT was measured both by functional and immunologic assays, with concordant results; PC was only measured immunologically. Hence, we cannot rule out the presence of low functional PC with normal or borderline antigenic PC. This possibility, however, appears unlikely because of the close correlation between antigenic PC and both functional and antigenic AT III.

Many previous studies have shown that PC and AT III are markedly reduced during DIC\textsuperscript{13,14,27,28} and in liver disease.\textsuperscript{14,27} When we compared leukemias with an overt laboratory picture of DIC with those without DIC, the PC and AT III levels were similar. Moreover, the inhibitors were no lower in patients with APL, a type of leukemia typically associated with severe DIC, than in those with other leukemias (AML and ALL) less frequently complicated by DIC. These findings must be taken as evidence that DIC is not involved in modifying the concentrations of PC and AT III in acute leukemias, which is at variance with other diseases complicated by DIC. In the last few years, a number of studies have suggested that the coagulopathy in acute leukemias might be due to mechanisms different from those in other forms of DIC. Egbring et al.\textsuperscript{29} for instance, have suggested that the release of proteases from leukemia blasts might be responsible for the destruction of fibrinogen and other coagulation factors, without necessarily involving an in vivo activation of blood coagulation with intravascular fibrin formation. The possibility that the coagulopathy in APL might be due to primary fibrinolysis must also be considered, because there has been a report of normal platelet survival and accelerated fibrinogen disappearance in this situation.\textsuperscript{30} However, recent data seem to negate the views that the coagulopathy in leukemias is

**Table 2. Comparison Between Hyperleukocytic and Nonhyperleukocytic Leukemias**

<table>
<thead>
<tr>
<th></th>
<th>Nonhyperleukocytic Leukemias (&lt;50,000/µl Blast Cells)</th>
<th>Hyperleukocytic Leukemias (&gt;50,000/µl Blast Cells)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein C (%)</td>
<td>79.8 (36–171)</td>
<td>57.0 (22–150)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Antithrombin III activity (%)</td>
<td>95.8 (58–156)</td>
<td>74.1 (59–92)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.92 (2.9–5.1)</td>
<td>3.32 (2.2–5.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pseudocholinesterase (mU/ml)</td>
<td>2,130 (1,106–4,094)</td>
<td>1,435 (854–2,412)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>200.9 (83–472)</td>
<td>206.3 (85–481)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values show means and 95% confidence limits (in parentheses).
not due to intravascular fibrin formation, because fibrinopeptide A, a fragment specifically cleaved from fibrinogen by thrombin, is markedly and consistently elevated in acute leukemias,\textsuperscript{31} and high levels can be quenched by heparin infusion.\textsuperscript{32} It seems, therefore, that DIC is the main cause of the coagulopathy occurring during acute leukemia, but that for unknown reasons, this form of DIC is not accompanied by consistent and severe reductions of PC and AT III plasma concentrations. The imbalance between nearly normal anticoagulant proteins and depressed levels of clotting factors and platelets might be one of the reasons why hemorrhages are clinically more prominent than thrombosis in leukemias complicated by DIC.

In search for an alternative mechanism to explain the low levels of PC and, to a lesser extent, of AT III seen in some leukemic patients, we found that there is a close correlation between the levels of these inhibitors and the synthetic function of the liver, as expressed by such indexes as serum albumin and pseudocholinesterase levels. In addition, both PC and AT III were lower in patients with the higher blood blast counts and who had more marked impairment of liver function, probably as a result of hepatic flow alterations leading to vascular damage and organ malfunction. These findings should be taken as evidence that low plasma levels of these inhibitors occur as a consequence of reduced synthesis by the liver. Griffin et al.\textsuperscript{33} also noted that, in patients with DIC, liver dysfunction alone was sufficient to account for low protein C antigen levels.

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