Clotting alterations in primary systemic amyloidosis

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ABSTRACT

Background and Objectives. The bleeding manifestations frequently observed in patients with immunoglobulin light chain amyloidosis (AL) have been attributed to different pathogenetic factors: amyloid deposits in several organs and systems leading to failures of these latter, the affinity of amyloid for some clotting factors, and the presence of plasma components interfering with fibrin formation could all induce alterations of clotting tests. This investigation aimed at defining the prevalence of clotting abnormalities and their clinical manifestations in patients with AL.

Design and Methods. Thirty-six consecutive patients with biopsy proven amyloidosis and documented monoclonal gammopathy were enrolled within one year. The following clotting tests were considered in the study: activated partial thromboplastin time (aPTT), prothrombin time (PT), thrombin time (TT), reptilase time (RT), Russell’s viper venom time (RVVT), fibrinogen, factor X and α2 antiplasmin.

Results. Hemorrhagic manifestations were mild to moderate in nine patients, but severe and untractable in one. The most frequent clotting anomaly was defective fibrinogen conversion to fibrin, as demonstrated by prolongation of both TT (85% of cases) and RT (90% of cases). Low levels of factor X activity were observed in about 1 out of 4 samples, while fibrinogen and α2 antiplasmin levels were distributed over a wide range of values. PT was prolonged in 8 and aPTT in 25 patients. The search for lupus anticoagulant was negative in samples showing a prolongation of aPTT and/or RVVT.

Interpretation and Conclusions. The prolongation of TT and RT is not dependent on either the presence of a heparin-like substance in the plasma or on fibrinogen levels; furthermore, the prolongation of RVVT is not related to factor X level. The hypothesized presence in the plasma of an inhibitor of fibrin formation could also affect factor X activation by Russell viper venom. The prolongation of TT and RT represents a peculiar feature of amyloidosis. The variability in the behavior of the other clotting times and hemostatic factors studied is mirrored in the heterogeneity of the clinical features observed in this disease.

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Key words: amyloidosis, hemostasis anomalies, clotting tests, bleeding tendency

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etechiae, ecchymoses and bleeding tendency are common clinical features in primary systemic amyloidosis (AL). At the onset of the disease, purpura, particularly in the periorbital and facial areas, is present in about 15% of patients. Furthermore severe hemorrhages, which are reported at diagnosis in only a few patients, may contribute to worsening the clinical course and lead to death. Hemostatic abnormalities are caused by several interacting pathogenic mechanisms. Amyloid deposits in peri-vascular regions, the presence of plasma inhibitors of fibrinogen conversion to fibrin, deficiencies of factor X, IX and X caused by their affinity for amyloid substance, and the presence of circulating heparin-like anticoagulants are all considered to play important roles in determining the hemostatic abnormalities. This study was aimed at investigating clotting abnormalities in 36 consecutive patients with amyloidosis.

Design and Methods

Patients

Thirty-six consecutive patients with amyloidosis proven by biopsy obtained from periumbilical fat aspiration were enrolled within one year in our Department of Internal Medicine, University of Pavia and IRCCS Policlinico San Matteo, Pavia. All patients gave informed consent to bone marrow aspiration in order to search for a monoclonal bone cell population, and to collection of blood samples in order to study coagulation abnormalities.

Characterization of monoclonal components

Serum and urine samples were collected at diagnosis. The light chain nature of amyloid deposits was inferred from detection of a monoclonal immunoglobulin in serum and urine through high resolution gel electrophoresis and immunofixation, or from the presence of a monoclonal bone marrow cell population revealed by immunofluorescence according to the technique described by Perfetti et al.

Coagulation studies

Venous blood samples were collected with a plastic syringe and dispensed into a plastic vial containing trisodium citrate and aprotinin (Trasyrol Bayer,
Leverkusen, Germany) at final concentrations of 0.38% and 260 KIU, respectively, stored on ice and immediately centrifuged at 4°C and 1,700 g for 10 min.

Platelet poor plasma was removed and frozen at −70°C until use. Coagulation studies were performed on all plasma samples utilizing the same lots of reagents. Venous blood samples obtained from 40 healthy volunteers were handled and analyzed with the same techniques and considered as reference values.

Clotting times included activated thromboplastin time (aPTT) (Pathoptin Behringwerke, Marburg, Germany), prothrombin time (PT) (Thromborel Ş- Behringwerke), thrombin time (TT) (Hemodiagnostica-Stago, Asnières, France), reptilase time (RT) (Hemodiagnostica-Stago), Russell viper venom time (RVVT) (Russell Viper Venom, Murex Diagnostic Ltd, Dartford, England) and were performed according to the manufacturers’ instructions. Fibrinogen was evaluated by the Clauss method (M ultifibrin-Behringwerke), factor X activity by the clotting method using factor X-deficient plasma as substrate (Stago-Deficient X, Asnières, France), α2-antiplasmin (α2AP) by a chromogenic technique (Behrichrome, α2 antiplasmin, Behringwerke). aPTT and RVVT were also measured in a 1:1 mixture of normal plasma and patient’s plasma for 30 patients with prolonged aPTT and/or RVVT in order to search for lupus anticoagulant (LA).

A result was considered to be abnormal when the patient’s plasma value was outside the reference ranges.

Statistical analysis
Basic statistics and linear regression analysis were performed using the Statistica program for PC.

Results
The clinical features of the patients studied are listed in Table 1. Hemorrhagic manifestations were mild to moderate, being chiefly characterized by purpura or ecchymoses in 9 patients. In only one patient was the main symptom severe untractable mucosal bleeding, which was the cause of his death.

In two patients a diagnosis of AL amyloidosis was made on a bone marrow cell population κ/λ ratio; lambda light chain was the isotype most frequently detected.

Prolonged clotting times were observed in a high percentage of the patients (Table 2). The most frequent abnormality was a defect of fibrinogen conversion to fibrin, as demonstrated by the prolongation of both thrombin and reptilase times which were significantly correlated (r = 0.81; p < 0.05). In 25 patients with a prolonged aPTT and in 5 patients with a prolonged RVVT, the search for lupus anticoagulant was negative. Low levels of factor X were observed in about 1 out of 4 samples, whereas plasma fibrinogen and α2AP levels were distributed over a wide range of values (Table 3). No relationships emerged between RVVT and factor X activity (r = 0.26), nor between fibrinogen levels and TT (r = 0.02) or RT (r = 0.29).

The patient who suffered from severe hemorrhages showed prolonged aPTT, TT, and RT, normal levels of factor X activity and fibrinogen, and low α2AP activity (54U/dL).

Discussion
Our results are in agreement with those reported by Gastineau et al.7 and confirm that prolonged thrombin and reptilase times are the most common coagulation abnormalities in AL amyloidosis. The serum monoclonal components did not affect these alterations since we observed the same defects even in sera with undetectable monoclonal protein. Since RT is not influenced by heparin, we can also exclude the presence of circulating heparin-like anticoagulant because thrombin and reptilase times were similarly altered. Abnormal fibrinogen and/or elevated fibrinogen/fibrin degradation products (FDP) are considered to be the main factors that affect both TT and RT. Dysfibrinogenemia was excluded from having a role in TT prolongation by Gastineau et al.,7 who demonstrated that fibrinogen purified from the plasma of AL patients was normally converted to fibrin by thrombin and that plasma collected from AL patients prolonged thrombin time on fibrinogen purified from normal

Table 1. Clinical data from 36 patients with amyloidosis.

<table>
<thead>
<tr>
<th>Trait</th>
<th>No. of pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (range of age 40-70 yrs)</td>
<td>12</td>
</tr>
<tr>
<td>Males (range of age 39-78 yrs)</td>
<td>24</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>9</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>8</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>7</td>
</tr>
<tr>
<td>Renal insufficiency (creatinine &gt;1.2 mg/dL)</td>
<td>9</td>
</tr>
<tr>
<td>Macroglossia</td>
<td>3</td>
</tr>
<tr>
<td>Gastrointestinal involvement</td>
<td>2</td>
</tr>
<tr>
<td>Peripheral neuropathies</td>
<td>6</td>
</tr>
<tr>
<td>Bleeding symptoms</td>
<td>10</td>
</tr>
<tr>
<td>History of past deep venous thrombosis (not on anticoagulant treatment)</td>
<td>3</td>
</tr>
<tr>
<td>IgG κ/ IgG λ</td>
<td>5/9</td>
</tr>
<tr>
<td>IgA κ/ IgA λ</td>
<td>1/1</td>
</tr>
<tr>
<td>Free κ/ free λ</td>
<td>5/12</td>
</tr>
<tr>
<td>None</td>
<td>2*</td>
</tr>
</tbody>
</table>

* Diagnosis performed on BMPC κ/λ ratio.

Table 2. Values of clotting times (sec) in 36 patients with AL amyloidosis.

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean ±SD</th>
<th>Range of Prolongation</th>
<th>Range of reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPTT</td>
<td>53.4±20.3</td>
<td>29-144</td>
<td>65</td>
</tr>
<tr>
<td>PT ratio</td>
<td>1.2±0.2</td>
<td>0.9-2.2</td>
<td>22</td>
</tr>
<tr>
<td>TT</td>
<td>16.3±2.9</td>
<td>11.8-23.3</td>
<td>85</td>
</tr>
<tr>
<td>RT</td>
<td>25.8±5.9</td>
<td>16.7-43.3</td>
<td>90</td>
</tr>
<tr>
<td>RVVT</td>
<td>39.4±11.6</td>
<td>26.4-63.8</td>
<td>89</td>
</tr>
</tbody>
</table>

aPTT = activated partial thromboplastin time, PT = prothrombin time, TT = thrombin time, RT = reptilase time, RVVT = Russell viper venom time.
deposits in the gastrointestinal tract, reduced food intake due to macroglossia or vomiting, liver failure due to hepatic cirrhosis, amyloid deposits in vascular walls, the gastrointestinal tract and liver should be taken into account in order to explain the abnormal hemostasis observed in these patients. Furthermore, our data suggest that a prolonged TT found in a coagulation screening test in a non-bleeding subject should make a differential diagnosis of amyloidosis be considered.

In conclusion, prolongation of TT and RT is a peculiar feature of amyloidosis, but it does not seem to affect bleeding manifestations. Other pathogenetic factors such as factor X deficiency, enhanced fibrinolysis, amyloid deposits in vascular walls, the gastrointestinal tract and liver should be taken into account in order to explain the abnormal hemostasis observed in these patients. Furthermore, our data suggest that a prolonged TT found in a coagulation screening test in a non-bleeding subject should make a differential diagnosis of amyloidosis be considered.

Contributions and Acknowledgments
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Funding
This work was supported in part by FAR 1997 grants from the University of Pavia and IRCCS Polidico San Matteo, Italy.

Disclosures
Conflict of interest: none.
Redundant publications: no substantial overlapping with previous papers.

Manuscript processing
Manuscript received July 30, 1999; accepted November 29, 1999.

Potential implications for clinical practice
- TT and RT prolongation is frequent and it can be present also in asymptomatic patients.
- A prolonged TT found in a coagulation screening test in a non-bleeding subject should make a differential diagnosis of amyloidosis be considered.
- Amyloidosis hemostatic defects are multifactorial and therefore their treatment needs to be multifaceted.

Table 3. Fibrinogen, factor X and α2 antiplasmin levels detected in our plasma samples.

<table>
<thead>
<tr>
<th>Test</th>
<th>M ±sd</th>
<th>Range</th>
<th>Abnormal results (%)</th>
<th>Range of reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>434±160</td>
<td>82-744</td>
<td>&lt;180 = 5</td>
<td>&gt;400 = 49</td>
</tr>
<tr>
<td>Factor X (U/dL)</td>
<td>85.9±26.3</td>
<td>40-150</td>
<td>&lt;65 = 27</td>
<td>72-155</td>
</tr>
<tr>
<td>α2 AP (U/dL)</td>
<td>97.8±30.1</td>
<td>46-137</td>
<td>&lt;65 = 17</td>
<td>70-146</td>
</tr>
</tbody>
</table>

α2 AP = α2 antiplasmin.

were detected in about 85% of our patients, clinical bleeding manifestations were almost absent (26 patients) or mild to moderate (9 patients). Autologous stem cell transplantation is currently offered to patients with AL amyloidosis; this procedure may be complicated by massive gastrointestinal hemorrhages caused by a worsening of imbalance between protease and antiprotease systems already reported in systemic amyloidosis. Excessive fibrinolysis, due to either elevated activation or defective inhibition has also been observed in amyloidosis. Enhanced fibrinolysis related to an α2AP deficiency probably contributed significantly to the severe blood losses in our patient with diffuse bleeding and worsened his clinical course.

In conclusion, prolongation of TT and RT is a peculiar feature of amyloidosis, but it does not seem to affect bleeding manifestations. Other pathogenetic factors such as factor X deficiency, enhanced fibrinolysis, amyloid deposits in vascular walls, the gastrointestinal tract and liver should be taken into account in order to explain the abnormal hemostasis observed in these patients. Furthermore, our data suggest that a prolonged TT found in a coagulation screening test in a non-bleeding subject should make a differential diagnosis of amyloidosis be considered.

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