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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 was 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 gammapathy 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 (RVTT), fibrinogen, factor X and α-2 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 alpha2 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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Petechiae, ecchymoses and bleeding tendency are common clinical features in primary systemic amyloidosis (AL).[1-3] At the onset of the disease, purpura, particularly in the periorbital and facial areas, is present in about 15% of patients.[4] Furthermore severe hemorrhages, which are reported at diagnosis in only a few patients,[4] may contribute to worsening the clinical course and lead to death.[2,5] Hemostatic abnormalities are caused by several interacting pathogenic mechanisms. Amyloid deposits in perivascular regions,[5,6] the presence of plasma inhibitors of fibrinogen conversion to fibrin,[7] deficiencies of factor X,[8-11] IX[12] and V[13] caused by their affinity for amyloid substance, and the presence of circulating heparin-like anticoagulants[14] 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.*[15]

Coagulation studies

Venous blood samples were collected with a plastic syringe and dispensed into a plastic vial containing trisodium citrate and aprotinin (Trasylol 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) (Pathromptin Behringwerke, Marburg, Germany), prothrombin time (PT) (Thromborel S-Behringwerke), thrombin time (TT) (Hemodiagnostica-Stago, Asnières, France), reptilase time (RT) (Hemodiagnostica-Stago), Russell viper venom time (RVTT) (Russell Viper Venom, Murex Diagnostic Ltd, Dartford, England) and were performed according to the manufacturers' instructions. Fibrinogen was evaluated by the Clauss method (Multifibren-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 α2 AP 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).

Table 1. Clinical data from 36 patients with amyloidosis.

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

**Diagnosis performed on BMPC κ/λ ratio.*

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

Test	*M±sd*	*Range*	*Prolongation %*	*Range of reference values*
aPTT	53.4±20.3	29-144	65	29.8-40.2
PT ratio	1.2±0.2	0.9-2.2	22	0.8-1.2
TT	16.3 ±2.9	11.8-23.3	85	10.2-13.1
RT	25.8±5.9	16.7-43.3	90	14.9-20.3
RVVT	39.4±11.6	26.4-63.8	89	24.3- 30.2

aPTT = activated partial thromboplastin time, PT = prothrombin time, TT = thrombin time, RT = reptilase time, RVVT = Russell viper venom time.

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

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

α2 AP = α2 antiplasmin.

subjects; furthermore, the addition of fibrin split products did not influence these results. On the basis of these observations, the authors concluded that inhibitors must exist in the plasma of patients with AL amyloidosis and that the inhibitory activity remains in the supernatant after fibrinogen is precipitated. In 10 out of the 33 patients with prolonged TT and/or RT enrolled in this study, FDP detected during hospitalization periods were in the normal range, and one patient showed FDP levels between 10 and 40 mg/L; in the others FDP were not tested. Furthermore no patient had a low platelet count which would have been suggestive of disseminated intravascular coagulation. In our opinion this plasma inhibitory activity on thrombin time might also influence RVVT, since we were not able to find any relationship between RVVT prolongation and FX activity. Hypofibrinogenemia, detected in only two patients, may be related to defective synthesis caused by liver failure[16] together with increased consumption. Markers of thrombin activation have been observed in AL patients even in the presence of a bleeding tendency;[17] in our previous report about half of the subjects with amyloidosis presented high levels of thrombin-antithrombin complexes,[18] and a history of past deep venous thromboses was present in three patients enrolled in this study. Our patients with low fibrinogen levels also had enhanced thrombin-antithrombin complexes,[18] suggesting increased fibrinogen turnover. On the other hand, the high fibrinogen levels detected in about 50% of the patients could have been an expression of enhanced acute phase proteins induced by abnormal production of cytokines, as is the case in multiple myeloma.[19]

LA, rarely observed in patients with monoclonal gammapathies,[20] has not yet been reported in AL amyloidosis. Furthermore, our research excluded a causative role for LA in the prolongation of aPTT and RVVT. Several pathologic conditions other than the presence of plasma thrombin time inhibitor could explain the prolongation of aPTT and PT in AL patients, e.g. malabsorption caused by amyloid deposits in the gastrointestinal tract,[21] reduced food intake due to macroglossia or vomiting,[4] liver failure[22] and plasma deficiencies of some clotting factors due to their affinity for amyloid deposits.[8-12]

Although alterations of one or more clotting tests 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.[23,24] Excessive fibrinolysis, due to either elevated activation[25] or defective inhibition[26,27] 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.

Contributions and Acknowledgments

GG conception and design, interpretation of data and critical revision; NM analysis of data; EA, GP, MC, ES, drafting the article and collection of data; GM final approval of the version to be published.

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Disclosures

Conflict of interest: none.

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Manuscript processing

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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.

References

1. Falk RH, Comenzo RI, Skinner M. The systemic amyloidoses. N Engl J Med 1997; 337:898-909.
2. Yood RA, Skinner M, Rubinow A, Talarico L, Cohen AS. Bleeding manifestations in 100 patients with amy-

loidosis. JAMA 1983; 249:1322-4.
3. Glaspy JA. Hemostatic abnormalities in multiple myeloma and related disorders. Hematol Oncol Clin N 1992; 6:1301-14.
4. Kyle RA, Gertz MA. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. Semin Hematol 1995; 32:45-59.
5. Kaiserling E, Krober S. Massive intestinal hemorrhage associated with intestinal amyloidosis. An investigation of underlying pathologic processes. Gen Diagn Pathol 1995; 141:147-54.
6. Hoshino Y, Hatake K, Muroi K, et al. Bleeding tendency caused by the deposit of amyloid substance in the perivascular region. Intern Med 1993; 32:879-81.
7. Gastineau DA, Gertz MA, Daniels TM, Kyle RA, Bowie EJ. Inhibitor of the thrombin time in systemic amyloidosis: a common coagulation abnormality. Blood 1991; 77:2637-40.
8. Furie B, Green E, Furie BC. Syndrome of acquired factor X deficiency and systemic amyloidosis in vivo studies of the metabolic fate of factor X. N Engl J Med 1977; 297:81-5.
9. Greipp PR, Kyle RA, Bowie EJ. Factor-X deficiency in amyloidosis: a critical review. Am J Hematol 1981; 11:443-50.
10. Furie B, Voo L, McAdam KP, Furie BC. Mechanism of factor X deficiency in systemic amyloidosis. N Engl J Med 1981; 304:827-30.
11. Skinner M, Talarico L, Cohen AS, St John A. The calcium dependent interaction of blood clotting factors with primary amyloid fibrils. In: Glenner GG, Costa PP, De Freitas AF, eds. Amyloid and amyloidosis. #497 of International Congress Series. Amsterdam: Excerpta Medica; 1980. p. 361-5.
12. McPherson RA, Onstad JW, Ugoretz RG, Wolf PL. Coagulopathy in amyloidosis: combined deficiency of factors IX and X. Am J Hematol 1977; 3:225-35.
13. Gatel A, Cacoub P, Piette JC. AL amyloidosis combined with acquired factor V deficiency. Ann Intern Med 1998; 128:604-5.
14. Khoory MS, Nesheim ME, Bowie EJ, Mann KG. Circulating heparan sulfate proteoglycan anticoagulant from a patient with a plasma cell disorder. J Clin Invest 1980; 65:666-74.
15. Perfetti V, Garini P, Colli Vignarelli M, Marinone MG, Zorzoli I, Merlini G. Diagnostic approach to and follow-up of difficult cases of AL amyloidosis. Haematologica 1995; 80:409-15.
16. Joist JH. Haemostatic abnormalities in liver diseases. In: Colman RW, Hirsh J, Marder VJ,Salzman EW, eds. Haemostasis and Thrombosis: Basic Principles and Clinical Practice. Philadelphia: JB Lippincott Company; 1994. p. 906-20.
17. Marcatti M, Mauri S, Tresoldi M, et al. Unusual bleeding manifestations in a case of primary amyloidosis with factor X deficiency but elevations of in vivo markers of thrombin formation and activity. Thromb Res 1995; 80: 333-7.
18. Gamba G, Montani N, Anesi E, Zorzoli I, Lorenzutti F, Merlini G. Alteration of the thrombin inactivation by antithrombin III may contribute to the abnormal haemostasis in AL amyloidosis. In: Kyle RA and Gertz MA, eds. Amyloid and Amyloidosis. New York: The Parthenon Publishing Group; 1999. p.145-7.
19. Nishimoto N, Shima Y, Yoshizaki K, Kishimoto T. Myeloma biology and therapy. Present status and future developments. Hematol Oncol Clin N 1997; 11:159-72.
20. Bellotti V, Gamba G, Merlini G, et al. Study of three patients with monoclonal gammopathies and 'lupus-like' anticoagulants. Br J Haematol 1989; 73:221-7.
21. Carlson HC, Breen JF. Amyloidosis and plasma cell dyscrasias: gastrointestinal involvement. Semin Roentgenol 1986; 21:128-38.
22. Gertz MA, Kyle RA. Hepatic amyloidosis (primary AL immunoglobulin light chain): the natural history in 80 patients. Am J Med 1988; 85:73-80.
23. Husby G, Stenstad T, Magnus JH, Sletten K, Nordvag BY, Marhaug G. Interaction between circulating amyloid fibril protein precursors and extracellular tissue matrix components in the pathogenesis of systemic amyloidosis. Clin Immunol Immunop 1994; 70:2-9.
24. Eriksson S, Janciauskiene S, Merlini G. The putative role of alpha-1-antitrypsin in the disaggregation of amyloid lambda fibrils. J Intern Med 1995; 237:143-9.
25. Liebman HA, Carfagano MK, Weitz IC, et al. Excessive fibrinolysis in amyloidosis associated with elevated plasma single-chain urokinase. Am J Pathol 1992; 98:534-41.
26. Meyer K, Williams EC. Fibrinolysis and acquired alpha-2 plasmin inhibitor deficiency in amyloidosis. Am J Med 1985; 79:394-6.
27. Takahashi H, Koike T, Yoshida N, et al. Excessive fibrinolysis in suspected amyloidosis: demonstration of plasmin-alpha 2-plasmin inhibitor complex and von Willebrand factor fragment in plasma. Am J Hematol 1986; 23:153-66.