Inhibitor of the Thrombin Time in Systemic Amyloidosis: A Common Coagulation Abnormality

By Dennis A. Gastineau, Morie A. Gertz, Todd M. Daniels, Robert A. Kyle, and E.J. Walter Bowie

Patients with primary systemic amyloidosis (AL) often experience bleeding, and we report a newly recognized coagulation abnormality in AL. Of 103 patients with primary systemic AL studied over 2 years, 41 had prolongation of the thrombin time (range, 25 to 46 seconds; normal, <22 seconds) and reptilase time (range, 17 to 39 seconds; normal, 14 to 16 seconds). The fibrinogen from the plasma of 36 patients was precipitated by β-alanine and diluted to a concentration of approximately 200 mg/dL. The thrombin times of the precipitated fibrinogens were normal in 34 patients, implying that an inhibitor was responsible for the abnormal tests. The addition of patient fibrinogen-free plasma to normal plasma prolonged the thrombin times, and this result confirmed the presence of an inhibitor. The inhibitor is more likely to be present in patients with nephrotic syndrome (20 of our patients) and congestive heart failure (six). A circulating monoclonal protein (24 patients), the presence of amyloid liver involvement (eight), and the presence of amyloid neuropathy (nine) were not predisposing factors. Only one patient had deficiency of factor X. We conclude that inhibition of fibrinogen conversion to a fibrin clot rather than dysfibrinogenemia is the cause of the prolonged thrombin time in primary systemic AL.

© 1991 by The American Society of Hematology.
plasma and allowed to incubate at room temperature for 30
minutes, and thrombin times were determined.

Plasma was depleted of fibrinogen by heating in a 56°C water-
bath for 4.5 minutes. Samples were centrifuged and tested for the
presence of inhibitor as described above.

*Interpretation.* The mean thrombin time of the normal PPP was
20.9 seconds (2 SD range, 17.7 to 24.1 seconds; 1 SD, 1.6 seconds;
n = 35). The mean thrombin time of the normal isolated fibrinogen
was 17.4 seconds (2 SD range, 14.9 to 19.9 seconds; 1 SD, 1.25
seconds; n = 35).

A normal result is a purified fibrinogen thrombin time less than
or equal to the normal PPP thrombin time (generally 18 to 21
seconds). A normal isolated fibrinogen thrombin time and pro-
longed PPP thrombin time are consistent with the presence of an
inhibitor. The prolongation of both clotting times is consistent with
dysfibrinogenemia.

**RESULTS**

The fibrinogen from the plasma of 36 patients with
abnormal thrombin times was precipitated by β-alanine and
diluted to a concentration of approximately 200 mg/dL. The thrombin
times of the precipitated fibrinogens were normal in 34 of the 36
patients, implying the presence of an inhibitor.

Three patients with known dysfibrinogenemia (one plasma
sample was kindly provided by Dr Douglas Triplett, Muncie, IN) were studied. The isolated fibrinogens of these
patients produced prolonged thrombin times (Fig 1). All were
substantially more prolonged than the only two amyloid-associated fibrinogens that did not correct to the normal range.

Normal and amyloid PPPs were depleted of fibrinogen
using 0.5% bentonite or heating to 56°C for 4.5 minutes. After
centrifugation, the supernatants were mixed with normal PPP and thrombin times were determined. Supernatant
from the three amyloid plasmas prolonged the thrombin
time (Table 1).

The thrombin time was the only coagulation test result
that was consistently abnormal: prolonged in 36 of the 41
patients studied (Table 2). Clottable fibrinogen levels
(biuret method) generally were elevated (range, 210 to
1,150 mg/dL; median, 636 mg/dL) in patients whose thrombin
time was prolonged. No patient had hypofibrinogen-
emia. Consistently negative protamine gel tests indicated
the absence of active disseminated intravascular coagulation.
The reptilase time was consistently elevated (range, 17
to 39 seconds; median, 24 seconds; control, 15 seconds) in
direct proportion to the prolongation of the thrombin time,
suggesting that a heparin-like inhibitor was not present.

The clinical abnormalities are listed in Table 3. The most
common findings were abnormal echocardiograms and
nephrotic syndrome. Although these were the most common,
no correlation with the degree of prolongation of the
thrombin time was found.

A circulating monoclonal protein was seen in 24 of the
patients. The results of immunoelectrophoresis are shown in
Table 4. The serum monoclonal protein was generally
small in amount and exceeded 1 g/dL in only nine of the
patients and exceeded 2 g/dL in only one. Nine patients had
a serum monoclonal protein and did not have associated
congestive heart failure or nephrotic syndrome. The thrombin
time in these nine patients ranged from 19.7 to 41.8
seconds (median, 22.3 seconds). In the five patients without
a monoclonal serum protein and without heart failure or
nephrotic syndrome, the thrombin time ranged from 20 to
26.3 seconds (median, 23.4 seconds). Only one patient had
a prolonged prothrombin time (22.2 seconds), and this
patient had a factor X level of 6%. The factor X levels were
not depressed below 40% in any of the other patients.

Figure 1 shows the results of the thrombin time before
and after the fibrinogen was purified. Of the 36 patients
whose thrombin time was prolonged 2 seconds greater than
control, the thrombin time normalized in 34. The thrombin
time was shortened a median of 8 seconds in the 36 whose
thrombin time was abnormal. In a control group of normals,
purification of the fibrinogen resulted in a median shorten-
ing of the thrombin time by 4 seconds.

Neither increasing the protein concentration with bovine
serum albumin nor adding fibrin split products (Dade)
resulted in prolongation of the thrombin time (data not
shown).

**Table 1. Mixing Studies to Demonstrate Inhibitor of Thrombin Time**

![Table 1](attachment:image)

---

**Fig 1. Thrombin times of PPP (○) and isolated fibrinogen (●) in
normals (n = 25), patients with dysfibrinogenemia (n = 3), and pa-
patients with AL (n = 36).**

For personal use only.on October 22, 2017. By request only.
DISCUSSION

The thrombin time can be prolonged for several reasons, including (1) the presence of heparin, (2) hypofibrinogenemia, (3) dysfibrinogenemia, (4) circulating anticoagulants, and (5) the presence of fibrin or fibrinogen split products. Differentiation of most of these causes is not difficult through the quantitative determination of the fibrinogen value and of the reptilase time to exclude heparin-type anticoagulants and through assays for fibrin degradation products and fibrin monomers.

However, generally dysfibrinogenemia is diagnosed through exclusion of other causes because no simple in vitro test exists to confirm the presence of a structurally abnormal fibrinogen. The cause of prolonged thrombin times in AL has been previously attributed to the presence of an abnormal fibrinogen. These studies report prolongation of prothrombin, activated partial thromboplastin, or thrombin times, but no mixing studies or purification of fibrinogen was reported.13,14

Bleeding in amyloidosis is multifactorial. Vascular infiltration of amyloid fibrils disrupts vascular integrity and has been associated with recurrent gastrointestinal hemorrhage.15 Coagulation factor deficiencies have been well established, and the apparent ability of amyloid fibrils to bind factor X has been described.6,7 Only one of our 41 patients had a prothrombin time greater than 2 seconds beyond control, and that patient had a factor X level of 6%: thus, factor X deficiency is rarely associated with an abnormal thrombin time. Severe liver involvement by amyloid causes deficiencies of many hepatically synthesized clotting factors and can imitate disseminated intravascular coagulation and fibrinolysis through failure of hepatic clearance of fibrin split products. Prolongation of the thrombin time in severe nonamyloid liver disease is due to abnormal fibrinogen sialic acid content resulting in abnormal fibrin monomer polymerization.16 Increased sialyltransferase activity produces a fibrinogen similar to fetal fibrinogen.57 Adding excess calcium characteristically shortens the thrombin time.

The presence of a serum monoclonal protein (usually associated with multiple myeloma) has been associated with heparin or heparin-like circulating anticoagulants.4 The prolongation of the thrombin time can be partially reversed with protamine. The thrombin time may also be shortened by the effects of chemotherapy, as we recently observed in a patient with a heparan-like inhibitor. These patients may experience severe bleeding, especially from the gastrointestinal tract.19

Our data suggest that the monoclonal protein in our patients did not cause the abnormal thrombin time because the thrombin times were not different between those amyloid patients with and those without a serum monoclonal protein. The thrombin time abnormality did not have any effect on the likelihood of clinical bleeding or thrombosis.

Hypofibrinogenemia may result from defective production of fibrinogen, as may be seen in severe liver disease.20 Total fibrinogen concentrations were preserved in our patients despite significant hepatomegaly in six. In fact, all patients tested had fibrinogen values greater than 200 mg/dL.

Circulating anticoagulants of the lupus-type were not specifically sought, and although some patients with monoclonal proteins have a lupus-type activity, these patients are rare, comprising only 10 of 219 such patients in our laboratory. None of these patients had amyloidosis.21

Butler and Baldwin6 described a patient with factor X deficiency who also had a prolonged thrombin time, and in a review of the literature they found that eight of 14 patients had either a thrombin time greater than 1.5 times control or a prolonged thrombin time that was not corrected in vitro when mixed with normal plasma. They postulated, as have others,2 that inhibitors may be present,
but they did not demonstrate direct evidence of inhibition or the presence of normal fibrinogen.

In this study we identified the presence of normal fibrinogen in patients who had AL with prolonged thrombin times and demonstrated the normal action of thrombin on this isolated fibrinogen. An inhibitor or inhibitors must exist in the plasma of patients with primary systemic AL. These inhibitors are separated by the fibrinogen isolation procedure.

The prolonged thrombin time in AL is not due to dysfibrinogenemia but to an inhibitory activity that remains in the supernatant after fibrinogen is precipitated.

REFERENCES

Inhibitor of the thrombin time in systemic amyloidosis: a common coagulation abnormality

DA Gastineau, MA Gertz, TM Daniels, RA Kyle and EJ Bowie