An acquired inhibitor that produced a delay of fibrinopeptide B release in an asymptomatic patient

An asymptomatic, 29-year-old woman was referred to our hospital before surgery because in the basic study of hemostasis she showed a prolonged thrombin time (TT) and a normal reptilase time (RT). She had not received any anticoagulants so, to account for these abnormal results the presence of an inhibitor or a dysfibrinogenemia was suspected. A 1:1 mixture of the patient's plasma with control plasma did not correct the TT. Dysfibrinogenemia was excluded because the defibrinated plasma retained the inhibitory activity when mixed with normal plasma. When 0.02 mg/ml of Protamine Sulphate (a concentration that neutralizes 1 U/mL of heparin in normal plasma) was added to the patient's plasma, the inhibitory activity did not disappear. IgG from the patient and from normal serum was isolated. The patient's IgG was able to prolong the TT of a normal plasma and of a purified fibrinogen. The patient IgG did not impair the catalytic activity of thrombin, because no difference was observed in the hydrolysis of S-2238 by 1 U NIH human thrombin with normal or patient IgG. The time course of the thrombin–mediated fibrinopeptide-release from normal fibrinogen with the patient's IgG, showed a delay in the fibrinopeptide B (FPB) release without affecting the fibrinopeptide A (FPA) release. This patient has an IgG antibody that delays fibrinopeptide B release of fibrinogen

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Case Report

An apparently healthy 29 years old woman without a tendency to bleed was referred to our center because of an abnormal coagulation test result that was detected prior to surgery for an ovarian cyst. The cyst was benign and was removed without any bleeding problems during or after the procedure. She had never been exposed to thrombin in fibrin glue. At present, she is healthy and asymptomatic.

Coagulation assays

In the routine coagulation screening APTT (activated partial thromboplastin time) and PT (prothrombin time) were in the normal range, but TT was prolonged in contrast with RT that was normal. The results of the coagulation test are shown in Table 1. No antiphospholipid antibodies were present. To determine if there were any other immunological abnormality, an immunological profile was performed. The results were normal and no monoclonal paraproteins were found (data not shown). The same results were obtained on 4 separated tests with different samples obtained over 2 years.

Inhibitor detection and characterization:

The prolonged TT with normal RT might be explained by the presence of heparin, but the patient did not receive heparin or any other anticoagulant. Also the samples had been properly obtained so a dysfibrinogenemia or an inhibitor of fibrin formation was suspected.

A 1:1 mixture of the patient's plasma with control plasma was incubated at 37 oC for 1 hour. This mixture exhibited a TT of 66 seconds in contrast to 21 seconds in the TT of control plasma and 122.8 seconds in the patient's plasma. These results suggested that an inhibitor was present in the patient's plasma. To confirm this, fibrinogen was precipitated from the patient's plasma by heat and the defibrinated plasma retained the inhibitory activity. The TT obtained in the patient's defibrinated plasma mixed with control plasma was 73.9 seconds versus 21.2 in control defibrinated plasma mixed with control plasma. These results indicated that the abnormality was not related to a defect in the fibrinogen molecule.

Table 1. Results of the Coagulation Tests

	Patient	*Normal range*
APTT (ratio)	1.23	0.75-1.3
PT (INR)	1.23	0.75-1.2
TT Bovine (s)*	39.9	17-24
TT Human (s)**	>60	17-24
RT (s)	21	16-23
Fg c (g/l)	1.91	1.5-4
Fg Ag (g/l)	2.34	1.5-4
D-Dimer (mg/ml)	<0.5	<0.5

*Abbreviations: INR: International Normalized Ratio, s: seconds, Fg c: coagulant fibrinogen, Fg Ag: antigen fibrinogen, *Thrombin IL ACL 9000, **Thrombin Sigma KC-10.*

There are reports[1-4] of patients who presented a heparin-like anticoagulant that prolonged the TT and could be neutralized by Protamine Sulphate (PS). To determine if this anticoagulant was heparin-like, we treated the patient's plasma with 0.02 mg/mL of PS that is the minimum concentration of PS that neutralized 1 U/mL of heparin in a control plasma. Control plasma with 1 U/mL of heparin and 0.02 mg/mL of PS gave a TT of 19.2 seconds. After addition of 0.02 mg/mL of PS to the patient and control plasma, the TT were 170 seconds and 19.2 seconds respectively. This indicated that the anticoagulant was not neutralized by PS and that it was not a heparin-like substance.

We proceeded to purified IgG from patient and control serum using a column of protein-G-sepharose. The patient's IgG when mixed with control plasma and compared with control IgG prolonged the TT from 29.3 to 66.1 seconds. The same results were obtained when IgG was mixed with purified fibrinogen. Patient and control IgG mixed with purified fibrinogen showed a TT of 95,6 and 62,5 seconds respectively. When IgG was removed from the patient's serum (serum without IgG) the inhibitory activity was lost (serum without IgG plus control plasma show a TT: 20.5 seconds). These results indicated that the inhibitor was an IgG antibody.

Effect of patient IgG on thrombin activity

The effect of the patient's IgG on thrombin activity was evaluated using the chromogenic substrate S-2238 for thrombin. No difference was observed in the substrate hydrolysis by 1 U NIH/mlL of human thrombin with normal IgG (residual thrombin obtained: 0.92 U/ml) or with the patient's IgG (residual thrombin obtained: 1.03 U/ml). This result indicated that the antibody had no effect upon the catalytic activity of thrombin.

Effect of patient IgG on fibrinopeptide release from fibrinogen

Despite the prolonged TT, the normal RT suggested

that the antibody might interfere with the release of FPB. To test this possibility, we performed a time-curve of fibrinopeptide release from fibrinogen in the presence of patient and control IgG : normal fibrinogen (0.1 mL at 2 mg/mL) was mixed with 0.1 mL at 4 mg/ml of purified patient or control IgG. The mixtures were incubated 60 minutes at 37°C, then, 20 mL of 10 NIH U/ml of human thrombin was added and the samples incubated at 37°C. The reaction was stopped at different times by incubating the samples at 100°C. The heat-precipitate was removed by centrifugation and the supernatant containing the fibrinopeptides were lyophilized. The concentrations of FPA and FPB released at each incubation time were analized by HPLC [5], the results are showed in Figure 1. When the patient's IgG was present, fibrinogen released less FPB than with the control IgG. In contrast, the FPA release was similar with patient or control IgG. The differences between FPB released from fibrinogen with control and with the patient's IgG were maximal at 1 to 2 minutes, and decreased with longer thrombin incubation times. Thus, it can be concluded that the patient's IgG produced a delay of FPB release from normal fibrinogen without affecting the FPA release. Figure 2 shows the algorithm of the diagnostic procedure.

Discussion

Acquired inhibitors that interfere directly with fibrin formation are rare and most of them occur in patients with autoimmune disease, malignancy or without underlying causes. Most of these inhibitors are related to heparin-like substances that appear from a disturbance of the endothelium. Others are related to a high paraprotein concentration that interferes with the polymerization of the fibrin monomers.[6-8] A third group of inhibitors consists of antibodies directed against the fibrinogen molecule[6, 9-13] probably at or near the binding sites of the thrombin. These inhibitors delay the fibrinopeptide A or B release. Our patient belongs to this last group. She had an IgG antibody that interfered with the FPB release from normal fibrinogen. This abnormality does not seem to be clinically relevant. Nawarawong[6] described a patient with an inhibitor that delayed FPB release and who did not present an abnormal bleeding history. Absence of a bleeding history has also been described in dysfibrinogenemias with delayed FPB release as the only abnormality.[14] Unlike the case described by Nawarawong,[6] our patient was healthy and did not exhibit any clinical or laboratory abnormalities.

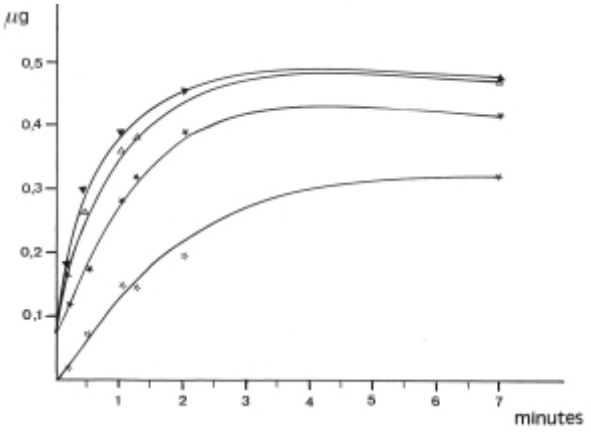

Figure 1. Release of FPA and FPB of Patient and Control. 0.1 ml of patient or control IgG (4mg/ml) were mixed with 0.1 ml of fibrinogen at 2 mg/ml, incubated 1 hour at 37°C and treated with 20 µl of 10 NIH U/ml of thrombin. The reaction was stopped at various times by heating at 100°C. The precipitate was removed by centrifugation and the supernatant lyophilized. These samples were dissolved in 100 µl of acetic acid, centrifuged for 4 minutes at 15000 g and finally 20µl from the supernatant were injected into the HPLC. Closed triangles show FPA released from samples with control IgG; open triangles show FPA released from samples with patient IgG; closed stars show FPB released from samples with control IgG and open stars show FPB released from samples with patient IgG

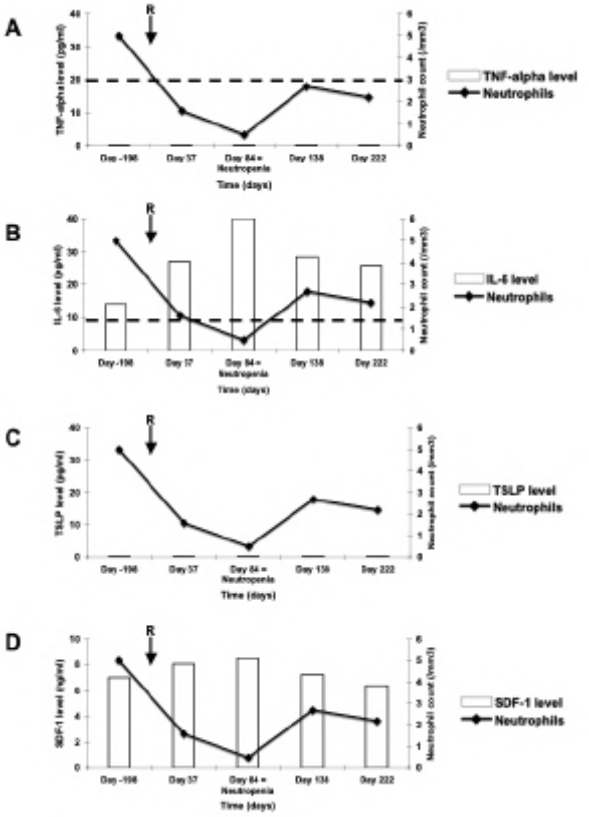

Figure 2. Algorithm chart of the diagnostic procure: Pp: patient plasma, Cp: control plasma, PS: protamine sulphate, Fg: fibrinogen, IgG: patient IgG, Thr: Thrombin, FP: Fibrinopeptide, FPB: Fibrinopeptide B.

D. Llobet, M. Borrell,* L. Vila,# C. Vallvé,**
R. Felices, J. Fontcuberta**
**Hemostasis Unit, Hematology Department; #Laboratory of Inflammation Mediators, Institute of Research. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.*

Correspondence: Montserrat Borrell
Hematology laboratory, Hospital de la Santa Creu i Sant Pau,
Avda. St Antoni Mª Claret 167. 08024 Barcelona, Spain.
E-mail: mborell@santpau.es
Tel: 34932919193 Fax: 34932919192
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