Platelet-Neutralization Procedure: Atypical Results Seen with Factor V Deficiency with and without an Inhibitor

DAVID L. MCGLASSON
RICHARD J. MERCIER
LYNNE F. POTEMPA
WAYNE R. PATTERSON

ABSTRACT: **A case of positive platelet-neutralization procedure (PNP) and abnormal mixing studies is presented.**

Atypical laboratory results were observed in a 55-year-old white man who had developed a spontaneous, acquired inhibitor against Factor V in response to treatment with cephalexin (Keflex, Lilly). The atypical results were obtained in a test that is usually specific for the presence of a lupus anticoagulant, PNP. PNP has remained positive in this patient for three years, and he continues to have a Factor V deficiency and inhibitor against Factor V.

A positive PNP test result is not specific for presence of a lupus anticoagulant but may be seen with a Factor V inhibitor or circumstances associated with any Factor V deficiency.

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Circulating anticoagulants are usually IgG or IgM immunoglobulins that are acquired inhibitors of hemostasis. They can either inactivate specific clotting factors or act as nonspecific anticoagulants that demonstrate inhibitory activity against certain anionic phospholipids.[1-3] Occasionally, patients with suspected lupus anticoagulants (LA) can produce laboratory data suggesting the presence of both specific and nonspecific circulating anticoagulants.[2]

The inhibitor occurring most frequently in specific coagulation deficiencies is directed against Factor VIII:C,[4] which occurs in 5–10% of patients with hemophilia A. This circulating anticoagulant can also occur in postpartum women and patients with drug reactions, autoimmune disorders, and certain malignancies.[5] Another spontaneously occurring inhibitor appears in approximately 3% of patients who have a Factor IX deficiency with hemophilia B.[1] Factor V inhibitor incidence is very low. Through 1986 there had been only 29 reported cases, usually associated with antibiotic therapy.[6] One other case in addition to the patient described below produced a Factor V inhibitor possibly related to cephalexin (Keflex, Lilly).[3]

Lupus anticoagulants have been described in a wide variety of clinical settings. They are rarely associated with bleeding but have been mentioned in connection with an increased risk of spontaneous abortion, microbiological infections, thrombosis, and autoimmune disorders. They inhibit the activation of prothrombin by directing their action against the procoagulant, phospholipid PF-3, in the prothrombin activator complex, (Xa, V, Ca^{2+}, PF-3).[7-11] In in vitro testing, the LA is one of the most common causes of a prolonged aPTT.[12] The patient described in this article displayed laboratory results that appeared to be caused by an LA indicated by a positive platelet-neutralization procedure (PNP) result and abnormal mixing studies with PT, aPTT, and kaolin-clotting time (KCT). Evidence for the presence of a Factor V deficiency with an inhibitor was demonstrated by decreased levels of Factor V activity and a specific Factor V inhibitor.

The presence of a positive PNP result suggestive of LA in the presence of decreased Factor V levels and a low Factor V inhibitor led us to question whether the atypical PNP results were caused by the presence of a specific inhibitor such as Factor V or a nonspecific inhibitor such as LA.

CASE REPORT

A 55-year-old white man with no prior history of bleeding developed a spontaneous Factor V deficiency with the presence of a strong inhibitor after he was treated with Keflex for a bacterial infection of the scrotum. No pretreatment baseline coagulation tests were available. Severe hemorrhaging occurred. Fresh frozen plasma failed to arrest the bleeding problem, which continued until platelet concentrates were added to his treatment regimen.

The initial coagulation tests revealed the following results: the prothrombin time (PT) was greater than 50 seconds; the aPTT was greater than 100 seconds. The thrombin time was 9.9 seconds for the patient with a control of 11.1 seconds. The fibrinogen level was 470 mg/dL with the fibrin split products (FSP) revealing a result greater than 80 μg/mL and less than 160 μg/mL. The platelet count was within normal limits at 207,000/μL. The coagulation mixing studies were abnormal. The patient's PT result was 60.8 seconds. The normal pool plasma was 10.5 seconds and the 1:1 mixing study corrected to 57.2 seconds. The patient's aPTT result was greater than 100 seconds. The normal pool plasma was 24.8 seconds and

David L. McGlasson, M.S., CLS/NCA, is in the Clinical Investigation Directorate; **Richard J. Mercier, M.D.,** is on staff, Hematology–Medical Oncology Service; **Lynne F. Potempa, MT (ASCP),** is in the Special Hematology Laboratory; and **Wayne R. Patterson, Ph.D., MT (ASCP),** is in the Clinical Investigations Directorate, Wilford Hall USAF Medical Center, Lackland Air Force Base, TX.
Address for reprints: David L. McGlasson, Clinical Investigation Directorate, Wilford Hall USAF Medical Center/SGS, Lackland AFB, TX 78236-5300.

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the 1:1 mixing study corrected to 87.2 seconds. After incubation at 37 °C for two hours the normal pool plasma aPTT was 30.1 seconds; however, the 1:1 mixing study aPTT had increased to greater than 200 seconds. KCT with normal pool plasma was 81.0 seconds. The patient's result was greater than 200 seconds. PNP was positive for the presence of LA. The baseline saline aPTT of the patient's plasma corrected from greater than 200 seconds to 94.1 seconds upon the substitution of the platelet concentrate for the saline. A greater than five-second correction of the baseline saline aPTT is suggestive of LA.

Coagulation factor assays revealed the virtual absence of Factor V. Factors II, VIII:C, IX, X, XI, and XII were all within normal limits. Further studies revealed a Factor V inhibitor of greater than 1200 Bethesda units.

After three years, the patient has continued to display a prolonged PT and aPTT with a low titer inhibitor of 0.5–1.67 Bethesda units with Factor V levels ranging from 0.04-0.08 U/mL. The PNP still suggests the presence of LA.

METHODS

Determination of PT, aPTT, and thrombin time were performed using standard laboratory procedures. The fibrinogen was performed by a modified thrombin time. FSPs were determined by latex agglutination. The factor assays were one-stage assays based upon PT (II, V, X) or aPTT (VIII, IX, XI, XII). The inhibitor activity was quantified in a manner analogous to Factor VIII inhibitors using a one-stage PT assay to detect residual Factor V activity.[13] KCT was performed in a manner described by Exner.[14,15] KCT is assayed using platelet-poor-plasma (PPP), which has a residual platelet count of less than 15,000/μL with no added phospholipid. Exner found that the KCT was more sensitive than the aPTT in a group of patients with systemic lupus erythematosus.

The PNP test was performed as advocated by Triplett.[16,17] PNP test is based on the ability of platelets to bypass the LA by significantly correcting a prolonged aPTT. Outdated blood bank platelets washed in Tris-buffered saline were frozen at −70 °C and stored until ready to use. The platelets were thawed and added to a patient plasma with a prolonged aPTT. Then a second aPTT was performed. If LA is present, a significant shortening of the aPTT occurs.[16]

The 1:1 inhibitor mixing studies for aPTT were run similarly to Brandt et al.[3] A commercially assayed lypholized reference plasma (ARP; Helena) with no evidence of inhibitor and a Factor V level of 0.9 U/mL was serially diluted with a known Factor V deficient plasma. A PNP test was run on each dilution.

RESULTS AND INTERPRETATION

The results of the initial coagulation tests after hospitalization for hemorrhaging are listed in the case report section. The prolonged PT and aPTT with a normal thrombin time and a lack of correction in regular mixing studies with normal plasma is indicative of a circulating anticoagulant. The prolongation of the 1:1 mixing study at 37 °C incubation with no correction of normal plasma is usually evidence of a temperature-enhanced specific factor inhibitor. However, there are reports of temperature-dependent LAs.[17] The KCT and PNP test showed evidence that LA was present on the initial studies as well as throughout the study. Coagulation Factor V was less than 0.01 U/mL on the initial samples. A reference laboratory identified a Factor V inhibitor that was greater than 1200 Bethesda units.

The ARP was serially diluted from 0.9 U/mL to 0.0 U/mL of Factor V. Then a PNP test was performed on each dilution (Table 1), displaying evidence that the PNP test can also be positive in the presence of a Factor V deficiency when no known circulating anticoagulant is present. Note that at a dilution of Factor V 0.225 U/mL, the PNP test displayed a positive result with a correction of 6.3 seconds. The dilution of Factor V 0.0 U/mL produced a correction of 82.6 seconds.

DISCUSSION

Brandt et al.[3] discussed a spontaneous Factor V inhibitor with unexpected laboratory features. The case that group presented very closely parallels our study. As they referenced and discussed, the combination of a prolonged PT and aPTT with a normal thrombin time and failure to correct the PT and aPTT in mixing studies in addition to a normal prothrombin-proconvertin time was indicative of the presence of a Factor V inhibitor.[18]

Our patient also had a PT that was greatly prolonged without evidence of an associated prothombin inhibitor. The prolongation of the incubated inhibitor screen aPTT at 37 °C with no correction of a 1:1 mixing study usually is indicative of a temperature-enhanced Factor VIII:C inhibitor. However in our study as well as the Brandt case, this was caused by the acquired Factor V inhibitor that was temperature enhanced.

The KCT test, a very sensitive procedure used to determine the presence of LAs, was also positive. However, it is not specific for the type of circulating anticoagulant present. The presence of heparin was ruled out by a normal thrombin time. The positive PNP results in this case also were noted in the Brandt study with a similar correction of the saline baseline aPTT using platelet concentrates. This correction of the PNP saline baseline aPTT corroborates previous studies that have shown that platelets contain substantial quantities of Factor V and cause shortening of the PT and aPTT in mixtures of Factor V inhibitor plasma and platelets.[19,20]

The Brandt study surmised that the positive PNP result in their case was probably

TABLE 1.
PLATELET-NEUTRALIZATION PROCEDURE (PNP) ASSAYED REFERENCE PLASMA (ARP) DILUTIONS OF FACTOR V

Factor V Levels (U/mL) in Diluted ARP[a]	Normal Saline ARP (aPTT) (sec)[a]	Platelet Concentrate ARP (aPTT) (sec)[a]	Correction (sec)
0.90	39.1	35.6	3.5
0.45	46.0	41.8	4.2
0.225	47.3	41.0	6.3[b]
0.113	50.7	43.9	6.8
0.057	58.6	46.9	11.7
0.029	74.2	57.4	16.8
0	172.9	90.3	82.6

[a] All dilutions of the ARP were made with a commercial Factor V-deficient plasma. The platelet concentration had a Factor V level of 0.06 U/mL of activity.

[b] Greater than five seconds correction of baseline saline aPTT is a positive PNP test.

caused by the low titer of the inhibitor in the plasma and the presence of Factor V in the platelet reagent required in the PNP. A previous study had cited the PNP test as being specific for identification of nonspecific inhibitors and giving negative results for Factor V, VIII:C, and IX inhibitors.[16] However, in our study, we demonstrated that the PNP test was positive in the presence of low Factor V activity whether or not an inhibitor was present.

CONCLUSION

The positive PNP test was evident in results achieved with the artificially prepared Factor V deficiency dilution of 0.225 U/mL. The final 0.0 U/mL dilution gave a result that corresponds closely to the PNP data as evidenced by the presence of a strong inhibitor. Our data provide further evidence that a positive PNP test result is not specific for the presence of LA but may be seen with a Factor V inhibitor or circumstances associated with any Factor V deficiency.

REFERENCES

1. Cassidy PG. Laboratory detection and characterization of circulating anticoagulant. Thromb Haemost No. TH 84-1 (TH-31).

2. Triplett DA. The clinical importance of lupus anticoagulants. Thromb Haemost No. TH 85-5 (TH-41).

3. Brandt JT, Britton A, Kraut EA. Spontaneous factor V inhibitor with unexpected laboratory features. Arch Pathol Lab Med 1986;110:224–7.

4. Shapiro SS. Antibodies to blood coagulation factors. Clin Haematol 1979;8:207–14.

5. Green D, Lechner K. A survey of 215 non-hemophiliac patients with inhibitors to factor VIII. Thromb Haemost 1981;45:200–3.

6. Nesheim ME, Nichols WL, Cole TL et al. Isolation and study of an acquired inhibitor of human coagulation Factor V. J Clin Invest 1986;77:405–15.

7. Rick ME, Hoyer LW. Haemostatic disorders in SLE. Clin Rheum Dis 1975;1:583–96.

8. Mueh JR, Hebst KD, Rappaport SI. Thrombosis in patients with the lupus anticoagulant. Ann Intern Med 1980;1982:156–9.

9. Shapiro SS, Thiagarajan P. Lupus anticoagulants. Prog Hemost Thromb 1982;6:263–85.

10. Canoso RT, Sise HJ. Chlorpromazine-induced lupus anticoagulant and associated immunologic abnormalities. Am J Hematol 1982;13:121–9.

11. Bajaj SP, Rappaport SI, Fimm DJ et al. A mechanism for the hypoprothrombinemia of the acquired hypoprothrombinemia-lupus anticoagulant syndrome. Blood 1983;61:684–92.

12. Kitchens CS. Prolonged activated partial thromboplastin time of unknown etiology. Am J Hematol 1988;23:38.

13. Kasper CK, Aledort IM, County RB et al. A more uniform measurement of Factor VIII inhibitors. Thromb Diath Haemorrh 1975;34:869–72.

14. Exner T, Rickard KA, Kronenberg H. A sensitive test demonstrating lupus anticoagulant and its behavioural patterns. Br J Haematol 1978;40:143–51.

15. Exner T. Comparison of two simple tests for the lupus anticoagulant. Am J Clin Pathol 1985;83:215–8.

16. Triplett DA, Brandt JT, Kaczor D et al. Laboratory diagnosis of lupus inhibitors: a comparison of the tissue thromboplastin inhibition procedure with a new platelet neutralization procedure. Am J Clin Pathol 1983;79:678–82.

17. Triplett DA, Brandt JT, Maas RL. The laboratory heterogeneity of lupus anticoagulants. Arch Pathol Lab Med 1985;109:946–51.

18. Feinstein DI. Acquired inhibitors of factor V. Thromb Haemost 1978;39:663–74.

19. Chediak J, Ashenhurst JB, Garlick I et al. Successful management of bleeding in a patient with factor V inhibitor by platelet transfusions. Blood 1980;56:835–41.

20. Chiu BHC, Rao DK, Becket C et al. Immune complexes containing factor V in a patient with an acquired neutralizing antibody. Blood 1985;65:810–8.