BRIEF OBSERVATION

THE AMERICAN JOURNAL of MEDICINE®

A Spontaneous Prothrombotic Disorder Resembling Heparin-induced Thrombocytopenia

Theodore E. Warkentin, MD,[a,b] Michael Makris, MD,[c] Richard M. Jay, MD,[d] John G. Kelton, MD[b]

[a]Department of Pathology and Molecular Medicine, [b]Department of Medicine, Michael G. DeGroote School of Medicine, McMaster University, Hamilton, Ontario, Canada; [c]Department of Haematology, Royal Hallamshire Hospital, Sheffield, United Kingdom; [d]Division of Medical Oncology and Clinical Haematology, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada.

ABSTRACT

BACKGROUND: Antibodies against the "self" protein, platelet factor 4 (PF4), bound to heparin—the cause of immune heparin-induced thrombocytopenia—are believed invariably to be triggered by preceding heparin therapy. We describe a novel syndrome, *spontaneous heparin-induced thrombocytopenia*, in which clinical and serologic features characteristic of this adverse drug reaction develop in patients despite the absence of preceding heparin therapy.

METHODS: Three patients met the study criteria (clinical and serologic features of heparin-induced thrombocytopenia without preceding heparin exposure), of whom 2 patients were identified among 225 patients (0.89%, 95% confidence interval, 0.11%-3.17%) with serologically confirmed heparin-induced thrombocytopenia recognized during an 18-year period at 1 hospital. The platelet serotonin-release assay was used to detect heparin-dependent immunoglobulin G-induced platelet activation, and 2 enzyme immunoassays were used to detect antibodies against PF4/heparin.

RESULTS: Two patients presented with thrombocytopenia and multiple arterial thrombosis, and 1 patient presented with anaphylactoid reactions after 2 subcutaneous injections of low-molecular-weight heparin. All 3 patients had high levels of platelet-activating anti-PF4/heparin antibodies of immunoglobulin G class at presentation despite the absence of previous heparin exposure. However, each patient did have a preceding infectious or inflammatory event; 1 patient had concomitant antiphospholipid antibodies.

CONCLUSION: Circumstances other than heparin use can trigger a spontaneous disorder that closely mimics heparin-induced thrombocytopenia, further supporting the autoimmune nature of this adverse drug reaction.

 • *The American Journal of Medicine (2008) 121, 632-636*

KEYWORDS: Heparin; Immunoglobulin G; Platelet; Prothrombotic; Thrombocytopenia

Heparin-induced thrombocytopenia is an acquired prothrombotic disorder.[1,2] The immune response targets platelet factor 4 (PF4), which forms neoepitopes when it binds to heparin.[3] The resulting immunoglobulin (Ig)G/PF4/heparin immune complexes activate platelets via their Fcγ receptors,[4] resulting in high thrombotic risk.[5,6] A central concept is that the antibodies are invariably triggered by preceding treatment with heparin, more often unfractionated compared with low-molecular-weight heparin.[1,2,6] On occasion, heparin-induced thrombocytopenia has autoimmune features. For example, thrombocytopenia and thrombosis can begin several days after all heparin has been stopped, so-called delayed-onset heparin-induced thrombocytopenia,[1,2] perhaps because unusually high levels of antibodies react with platelet-associated PF4 bound to non-heparin glycosaminoglycans (chondroitin sulfate).[7] In theory, such a disorder might even arise spontaneously, presenting as de novo thrombocytopenia and thrombosis or rapid-onset thrombocytopenia with anaphylactoid reactions on first heparin use. We report 3 patients whose clinical profiles support these concepts.

Grant support by the Heart and Stroke Foundation of Ontario (T-5207, T-6157 to Dr Warkentin and T-5542 to Dr Kelton).

Requests for reprints should be addressed to Ted Warkentin, MD, Room 1-180A, Hamilton Regional Laboratory Medicine Program, Hamilton Health Sciences (Hamilton General Site), 237 Barton Street East, Hamilton, Ontario L8L 2X2, Canada.

E-mail address: twarken@mcmaster.ca

0002-9343/$ -see front matter
doi:10.1016/j.amjmed.2008.03.012

CASE SUMMARIES

Approval was obtained from the surviving patients to report their cases and from the research ethics board for reporting the remaining case. Patients 1 and 2 were identified at the hospital of the primary author; patient 3 was identified through personal communication with Michael Makris, MD (January 2006).

Patient 1

A 69-year-old man without previous hospitalization presented with bilateral lower limb ischemia caused by iliac/femoral artery thromboses and thrombocytopenia (platelet count, 17×10^9/L). The only remarkable preceding events were a 5-day course of ampicillin for periodontitis and an intra-articular injection of methylprednisolone into his wrist 8 days earlier after local injury. Despite undergoing thrombectomies and receiving ancrod (defibrinogenating agent), lower limb necrosis developed. Fatal cardiac arrest occurred 2 hours after the amputations. Autopsy revealed multiple thrombi (coronary and lower limb arteries, pulmonary embolism, and hepatic vein thromboses). No neoplasm was found.

Patient 2

A 40-year-old woman without previous hospitalization developed sudden left-sided hemiparesis as the result of cerebral infarction (right middle cerebral artery thrombosis) (Figure 1A). The only remarkable recent events were several outpatient incision-and-drainage procedures for groin cysts (without heparin use). The platelet count was 297×10^9/L at the time of her stroke but decreased during the next few hours to 175×10^9/L, at which time symptomatic acute femoral artery thrombosis occurred. Embolectomy was performed, and heparin was intravenously administered intraoperatively and postoperatively, with further platelet count decreases (nadir, 59×10^9/L). Heparin was stopped, and neurosurgery was performed for cerebral edema. The alternative anticoagulant, danaparoid, was given postoperatively, with platelet count recovery.

Patient 3

A 24-year-old woman was admitted with a 2-week history of an upper respiratory tract infection and a 1-week history of productive cough and pleuritic chest pain; pneumonia was diagnosed (rales, chest x-ray findings) (Figure 1B). There was no previous hospitalization or history of heparin exposure. The baseline platelet count was 301×10^9/L. However, pending investigation for pulmonary embolism, she received enoxaparin (1 mg/kg) by subcutaneous injection. Within 30 minutes, she developed shaking chills, tachycardia, cyanosis, hypoxemia, and flushing, which subsided by 2 hours. (No repeat platelet count was measured at this time). The patient received a second subcutaneous dose of enoxaparin 43 hours later, with recurrence of these symptoms and signs. After this second reaction, the platelet count measured 62×10^9/L. Although computed tomography pulmonary angiography ruled out pulmonary embolism, danaparoid was administered pending platelet count recovery.

CLINICAL SIGNIFICANCE

- A disorder characterized by the clinical and serologic features of immune heparin-induced thrombocytopenia can occur in the absence of preceding heparin therapy ("spontaneous heparin-induced thrombocytopenia").
- The existence of spontaneous heparin-induced thrombocytopenia provides further evidence supporting the autoimmune nature of this adverse drug reaction.

MATERIALS AND METHODS

Inclusion criteria included patients meeting clinical and serologic criteria for heparin-induced thrombocytopenia[1,5,6] in whom preceding heparin therapy was neither identified nor plausible. Patient sera were tested for heparin-dependent platelet-activating IgG using the platelet serotonin-release assay.[8] A platelet Fc receptor-blocking monoclonal antibody was used to demonstrate that platelet activation occurred through Fcγ receptors.[4] Two enzyme immunoassays were used to detect antibodies against PF4/heparin: an in-house assay[3] that detects IgG and a commercial enzyme immunoassay that detects IgG, IgA, and IgM class antibodies against PF4/polyvinylsulfonate.[9] For the 2 patients who received heparin, the sera tested were obtained before heparin exposure (patient 2) or within 20 minutes after starting heparin (patient 3). Follow-up sera also were tested in the 2 surviving patients. Because 2 cases were observed at 1 hospital (during 1992 and 2002), we reviewed the laboratory records to determine the total number of patients diagnosed at that hospital with serologically confirmed heparin-induced thrombocytopenia (by serotonin-release assay) over the 18-year period during which the primary author worked there.

Testing for antiphospholipid antibodies included enzyme immunoassays for IgM and IgG anticardiolipin and anti-β_2 glycoprotein-1 antibodies (QUANTA Lite; INOVA Diagnostics, San Diego, Calif), and testing for nonspecific inhibitor (PTT-LA, Diagnostica Stago, Asnieres, France; dilute Russell viper venom time, LA Check, with confirmatory step [LA Sure], PrecisionBioLogic, Dartmouth, Nova Scotia).

RESULTS

The 3 patients showed positive test results for heparin-induced thrombocytopenia antibodies by both the serotonin-release assay and the 2 enzyme immunoassays (Table 1). Testing using blood obtained 36 months (patient 2) and 7 months (patient 3) later showed negative results. Only 1

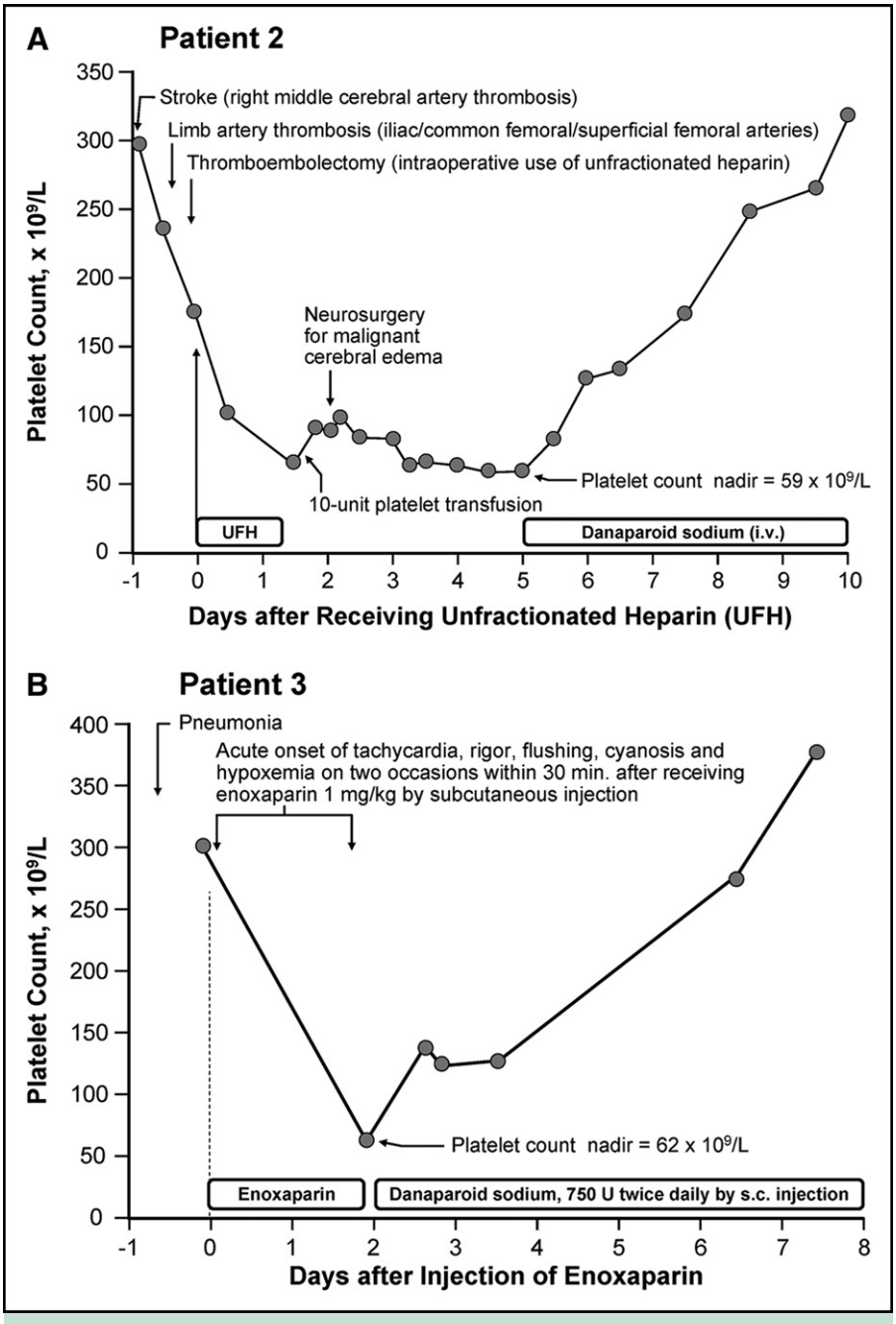

Figure 1 A, Clinical course of patient 2. B, Clinical course of patient 3; UFH = unfractionated heparin; iv = intravenously; sc = subcutaneously.

patient showed positive test results for antiphospholipid antibodies, with negative test results at the 36-month follow-up. "Spontaneous heparin-induced thrombocytopenia" represented 0.89% (95% confidence interval, 0.11%-3.17%) of patients diagnosed with heparin-induced thrombocytopenia at 1 hospital site.

DISCUSSION

We describe 3 patients with clinical and serologic features of heparin-induced thrombocytopenia despite the absence of preceding heparin exposure. Two patients presented with thrombocytopenia and thrombosis, with events typical of heparin-induced thrombocytopenia (limb artery thrombosis, stroke, and venous thromboembolism).[1,2,5,6] One patient died; the other had a debilitating stroke and limb amputation.

The third patient, who did not present with thrombocytopenia or thrombosis, developed anaphylactoid reactions on 2 occasions shortly after receiving 2 injections of low-molecular-weight heparin, with an associated platelet count decrease. Similar reactions with abrupt platelet count decreases after intravenous bolus heparin[10] or subcutaneous

Table 1 Serologic Features of 3 Patients with Platelet-activating Anti-PF4/Heparin Antibodies in the Absence of Preceding Heparin Therapy

Patient No. (Age, Sex)	Platelet Activation Assay for Heparin-induced Thrombocytopenia (Percent Serotonin Release)*			Enzyme Immunoassays to Detect Anti-PF4/Heparin Antibodies† (Absorbance, Optical Density Units)		Tests for Antiphospholipid Antibodies			
	0 IU/mL UFH (Normal < 10%)	0.1 IU/mL UFH (Normal < 10%)	0.3 IU/mL UFH (Normal < 10%)	In-house Assay that Detects IgG Anti-PF4/Heparin (Normal < 0.45 U)	Commercial Assay that Detects IgG/IgA/IgM Anti-PF4/Polyvinyl Sulfonate Antibodies (Normal < 0.40 U)	Anticardiolipin and Anti-β_2-Glycoprotein-1 IgM and IgG Antibodies	PTT-LA	Dilute RVVT (with Confirmatory Step)	Interpretation
1 (69, M)	47	99	96	2.03	2.72	Negative	Negative	Negative	Normal
2 (40, F)	76	91	95	2.91	2.28	Positive‡	Negative	Positive	Antiphospholipid antibodies
3 (24, F)	0	37	80	1.87	2.62	Negative	Negative	Negative	Normal

PF4 = platelet factor 4; PTT-LA = partial thromboplastin time-lupus anticoagulant; RVVT = Russell viper venom time; UFH = unfractionated heparin. For all assays, testing of negative and positive controls reacted as expected. Testing for antibodies was performed for patient 3 using a blood sample obtained 20 minutes after the first injection of enoxaparin (earlier blood samples were not available); for patient 2, the blood tested was obtained before any administration of heparin (patient 1 never received heparin).

*For all patients, reactivity was inhibited to less than 2% in the presence of either Fcγ receptor blocking monoclonal antibody or high concentrations (100 IU/mL) of UFH.

†For all patients, reactivity in both enzyme immunoassays was inhibited by more than 80% in the presence of UFH, 100 IU/mL, which is a characteristic feature of antibodies causing heparin-induced thrombocytopenia.

‡Low-titer positive reactions in both IgM and IgG anticardiolipin enzyme immunoassays, but negative tests in both IgM and IgG anti-β2-glycoprotein-1 enzyme immunoassays.

low-molecular-weight heparin injections[11] are features of otherwise typical heparin-induced thrombocytopenia. In addition, the platelet count decreases observed in the 2 patients who received heparin also are consistent with heparin-induced thrombocytopenia.[5]

Each patient had a serologic profile characteristic of heparin-induced thrombocytopenia, with strong positive test results by 3 different assays (Table 1). Additional characteristic serologic features included inhibition of reactivity at high heparin concentrations[1,4,8] and of platelet activation by Fcγ receptor blockade.[4] The 2 survivors later had negative test results for heparin-dependent antibodies during follow-up, reflecting the transient nature of the anti-PF4/heparin immune response.[5]

Heparin-induced thrombocytopenia has several features consistent with an autoimmune pathogenesis. First, the antibodies bind to PF4, a self-protein. Second, the role of pharmacologic heparin in producing antigenic modifications on PF4 can be substituted for by endogenous heparan or chondroitin sulfate.[7,9] Third, patients with delayed-onset heparin-induced thrombocytopenia develop thrombocytopenia and thrombosis beginning several days after all heparin has been stopped;[1,2] sera from such patients contain antibodies that cause substantial platelet activation even in the absence of heparin.[1] Indeed, such a reaction profile was seen in patients 1 and 2, who presented with thrombocytopenia and thrombosis (Table 1).

An intriguing observation is that the 3 patients had infectious or inflammatory events during the 2-week interval preceding their acute illness. It is known that heparin is more immunogenic and more likely to cause immune thrombocytopenia when given to postsurgery, compared with medical, patients.[12] Perhaps proinflammatory factors potentiate the anti-PF4/heparin immune response, which rarely can be triggered without pharmacologic heparin. Only 1 patient had evidence of concomitant antiphospholipid antibodies. Although the "spontaneous" presence of anti-PF4/heparin antibodies despite the absence of previous heparin therapy has been observed in the antiphospholipid syndrome,[13] none of these patients had platelet-activating antibodies (unlike our patient). Moreover, the converse situation we are reporting (in 2 patients), namely, the spontaneous formation of platelet-activating, heparin-dependent antibodies *without* concomitant antiphospholipid antibodies, has not to our knowledge been reported.

CONCLUSIONS

We report 3 patients who developed an acute illness resembling heparin-induced thrombocytopenia despite the absence of preceding heparin exposure. This suggests that on rare occasions a transient prothrombotic disorder can occur that is characterized by the spontaneous formation of platelet-activating anti-PF4/heparin antibodies resembling those formed in heparin-induced thrombocytopenia.

ACKNOWLEDGMENTS

We thank Jane C. Moore and Jo-Ann I. Sheppard for technical assistance, and Jo-Ann I. Sheppard for preparing Figure 1.

References

1. Warkentin TE, Kelton JG. Delayed-onset heparin-induced thrombocytopenia and thrombosis. *Ann Intern Med*. 2001;135:502-506.
2. Rice L, Attisha WK, Drexler A, Francis JL. Delayed-onset heparin-induced thrombocytopenia. *Ann Intern Med*. 2002;136:210-215.
3. Horsewood P, Warkentin TE, Hayward CPM, Kelton JG. The epitope specificity of heparin-induced thrombocytopenia. *Br J Haematol*. 1996;95:161-167.
4. Kelton JG, Sheridan D, Santos A, et al. Heparin-induced thrombocytopenia: laboratory studies. *Blood*. 1988;72:925-930.
5. Warkentin TE, Kelton JG. Temporal aspects of heparin-induced thrombocytopenia. *N Engl J Med*. 2001;344:1286-1292.
6. Warkentin TE, Levine MN, Hirsh J, et al. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. *N Engl J Med*. 1995;332:1330-1335.
7. Rauova L, Zhai L, Kowalska MA, et al. Role of platelet surface PF4 antigenic complexes in heparin-induced thrombocytopenia pathogenesis: diagnostic and therapeutic implications. *Blood*. 2006;107:2346-2353.
8. Sheridan D, Carter C, Kelton JG. A diagnostic test for heparin-induced thrombocytopenia. *Blood*. 1986;67:27-30.
9. Visentin GP, Moghaddam M, Beery SE, et al. Heparin is not required for detection of antibodies associated with heparin-induced thrombocytopenia/thrombosis. *J Lab Clin Med*. 2001;138:22-31.
10. Mims MP, Manian P, Rice R. Acute cardiorespiratory collapse from heparin: a consequence of heparin-induced thrombocytopenia. *Eur J Haematol*. 2004;72:366-369.
11. Srinivasan AF, Rice L, Bartholomew JR, et al. Warfarin-induced skin necrosis and venous limb gangrene in the setting of heparin-induced thrombocytopenia. *Arch Intern Med*. 2004;164:66-70.
12. Warkentin TE, Sheppard JI, Sigouin CS, et al. Gender imbalance and risk factor interactions in heparin-induced thrombocytopenia. *Blood*. 2006;108:2937-2941.
13. Martinuzzo ME, Forastiero RR, Adamczuk Y, et al. Antiplatelet factor 4-heparin antibodies in patients with antiphospholipid antibodies. *Thromb Res*. 1999;95:271-279.