Spontaneous heparin-induced thrombocytopenia syndrome:
two new cases and a proposal for defining this disorder

Theodore E. Warkentin,¹ Paul A. Basciano,² Jared Knopman,³ and
Richard A. Bernstein⁴

Departments of ¹Pathology and Molecular Medicine, Michael G. DeGroote School of
Medicine, McMaster University, Hamilton, ON; and ²Department of Medicine, Weill
Cornell Medical Center, New York, NY, and ³Division of Interventional Neuroradiology,
Department of Neurosurgery, Weill Cornell Medical Center, New York, NY; and ⁴Ken
and Ruth Davee Department of Neurology and Clinical Neurological Sciences, Feinberg
School of Medicine of Northwestern University, Chicago, IL, USA.

Brief Report

Running title: Spontaneous HIT syndrome

Corresponding Author: Theodore (Ted) E. Warkentin, M.D.

Hamilton Regional Laboratory Medicine, Hamilton Health Sciences, Hamilton General
Site, Rm 1-270B, 237 Barton St. E., Hamilton, ON, L8L 2X2.

Phone: 905-527-0271 ext. 46139. Fax: 905-577-1421. Email: twarken@mcmaster.ca
Key Points

* Two well-documented cases of a HIT-mimicking disorder without proximate heparin exposure (spontaneous HIT syndrome) are reported
* The definition of spontaneous HIT syndrome should include strong serum-induced platelet activation at 0 IU/mL heparin (inhibited at 100 IU/mL)

Abstract

The existence of “spontaneous” heparin-induced thrombocytopenia (HIT) syndrome (or “autoimmune HIT”), defined as a transient prothrombotic thrombocytopenic disorder without proximate heparin exposure serologically indistinguishable from HIT, is controversial. We describe two new cases presenting with thrombotic stroke/thrombocytopenia, one following shoulder hemi-arthroplasty (performed without heparin), and the other presenting to the emergency room without prior hospitalization, heparin exposure or preceding infection. Both patients tested strongly positive for anti-PF4/heparin IgG in two different immunoassays, and in the platelet serotonin-release assay. Crucially, both patients’ sera also caused strong (>80%) serotonin-release in the absence of heparin, a serologic feature characteristic of “delayed-onset” HIT (i.e., where heparin use precedes HIT but is not required for subsequent development of thrombocytopenia). We propose that a rigorous definition of spontaneous HIT syndrome should include otherwise unexplained thrombocytopenia/thrombosis without proximate heparin exposure, and with anti-PF4/heparin IgG antibodies that cause strong in-vitro platelet activation even in the absence of heparin.
Introduction

Heparin-induced thrombocytopenia (HIT) is a transient, autoimmune-like, prothrombotic disorder caused by platelet-activating IgG reactive against the self protein, platelet factor 4 (PF4) bound to heparin.1,2 Rarely, a brief exposure to heparin triggers this adverse reaction beginning after stopping heparin (“delayed-onset HIT”); such patients’ sera activate platelets strongly in-vitro even in the absence of pharmacologic heparin.3 Previous reports4-7 suggest that an analogous syndrome can occur without preceding heparin exposure (“spontaneous” HIT syndrome or “autoimmune” HIT). We now report two new cases of spontaneous HIT syndrome, review the literature, and propose a clinical-pathological definition of this disorder including characteristic in-vitro platelet activation features. We also suggest that the existence of spontaneous HIT syndrome following orthopedic surgery confounds interpretation of fondaparinux-associated HIT reported in that patient population.8-12

Case Reports and Methods

Patient 1: A 62-year-old man was admitted with left middle cerebral artery (MCA) thrombotic stroke and thrombocytopenia; his admission platelet count (measured prior to receiving heparin) was 65×10⁹/L (Figs. 1A/B). There was no previous history of thrombocytopenia, hospitalization/surgery, previous heparin, infection, or other recent acute illness. MCA recanalization was initially achieved (Fig. 1C) by mechanical thrombectomy (Solitaire stent-retriever) with unfractionated heparin (UFH, 1000 IU plus an unspecified amount as heparinized saline infusion), but five rapid episodes of re-thrombosis (Fig. 1D) required five further passes with the Solitaire device. Finally, patency (Fig. 1E) was established with tissue-plasminogen activator (15 mg)
administered directly into the internal carotid artery terminus. A blood sample obtained prior to heparin administration tested strongly positive for HIT antibodies (see Results and Discussion). Treatment with aspirin, fondaparinux and argatroban was administered, with subsequent warfarin. The platelet count remained low for one week (nadir, 27×10⁹/L), recovering by day 14.

**Patient 2**: A 54-year-old female developed right leg swelling, left-upper extremity weakness/paresthesias, and thrombocytopenia (61×10⁹/L) 15 days post-shoulder hemiarthroplasty; no intra-/postoperative heparin had been given, and no central/invasive lines were used. Brain MRI demonstrated acute infarct in left posterior inferior cerebellar artery territory; angiography showed non-visualization of the left vertebral artery (V2 segment). Ultrasound venography showed right lower-extremity deep-vein thrombosis. Echocardiographic saline-bubble contrast study revealed no abnormalities. UFH treatment resulted in further platelet count fall to 37×10⁹/L (nadir). Subsequent treatment with argatroban followed by fondaparinux was associated with gradual platelet count recovery to >150×10⁹/L by day 39.

Patient/control sera were tested for PF4-dependent antibodies using three enzyme-immunoassays (EIAs): an in-house IgG-specific EIA¹³ and two commercial (Immucor GTI Diagnostics, Waukesha, WI)—one IgG-specific (“Lifecodes PF4 IgG”),¹³ the other a polyspecific EIA detecting IgG/IgA/IgM (“Lifecodes PF4 Enhanced”).¹⁴ We also performed the platelet serotonin-release assay (SRA), as described.¹⁵ Both patients provided informed consent to report their cases. This study was conducted in accordance with the Declaration of Helsinki.
Results and Discussion

Both patients’ admission sera tested strongly-positive for HIT antibodies by all three EIAs, and in the SRA. Moreover, both patients’ admission sera (obtained before heparin administration) caused strong platelet activation at 0.1 and 0.3 IU/mL UFH (100% serotonin-release), as well as in the absence of heparin (>80% serotonin-release), with no platelet activation at 100 IU/mL heparin (Table 1). Heparin-dependent platelet activation was confirmed using diluted patient sera (see Table legend). Similar serological features have been reported for patients with delayed-onset HIT as well as in our initial report describing spontaneous HIT syndrome. Antibody reactivity declined markedly by two to four weeks (including disappearance of platelet-activating properties at 0 IU/mL heparin), in keeping with the usual transience of HIT antibodies, and paralleling both patients’ platelet count recovery. Laboratory testing did not support a diagnosis of antiphospholipid syndrome (APS) in either patient (see Table legend) (both APS and spontaneous HIT syndrome are potential explanations for “thrombotic storm”).

These two cases further support spontaneous HIT syndrome as an unusual explanation for acute thrombosis and thrombocytopenia. Preceding orthopedic surgery is a possible trigger of these antibodies, as per patient 2 (present report) and as previously reported. Indeed, of the seven previously reported cases of spontaneous HIT syndrome in which patients presented with thrombocytopenia and thrombosis in the absence of proximate heparin exposure, five occurred post-orthopedic surgery (all with warfarin anticoagulation given post-knee replacement surgery), whereas the remaining two cases occurred post-infection. In patient 1 (present report), however, we could not identify a potentially causal proximate event. Including our two new cases (present report), plus
two previously reported cases (patients 1 and 2 in our initial report\(^4\)), there have now been four patients with putative spontaneous HIT syndrome investigated in the McMaster Platelet Immunology Laboratory. All four sera yielded substantial platelet activation (mean, 76\%) in the absence of pharmacologic heparin. This is a crucial observation, as it may explain how anti-PF\(_4\)/heparin antibodies can induce intravascular platelet activation at a time when no heparin is being administered.

Spontaneous HIT syndrome should not be confused with a related clinical picture resembling rapid-onset HIT triggered by therapeutic-dose heparin administration in which, however, no preceding heparin exposure can be identified, yet the patients have circulating anti-PF\(_4\)/heparin antibodies. This situation has been documented in two patients\(^4,18\) (without previous heparin exposure) who initially presented with normal platelet counts but then developed acute thrombocytopenia upon receiving therapeutic-dose low-molecular-weight heparin\(^4\) or UFH.\(^18\) Importantly, neither serum caused platelet activation at 0 IU/mL heparin, consistent with serum-induced platelet activation that occurs in the absence of heparin (i.e., at buffer control) being important in explaining spontaneous HIT syndrome that presents with thrombocytopenia.

We believe our observations could also have relevance to the understanding of the pathogenesis of fondaparinux-associated HIT. To date, six relatively well-documented cases of HIT following fondaparinux thromboprophylaxis have been reported,\(^8\)\(^-\)\(^12\) without any apparent preceding heparin exposure. Notably, five of the six patients had preceding orthopedic surgery (all but one post-knee replacement), raising the possibility that fondaparinux could be an innocent bystander, i.e., the orthopedic surgery itself could have been the trigger of the subsequent anti-PF\(_4\)/heparin immune response. Indeed, only
minor enhancement of platelet activation in-vitro in the presence of fondaparinux has been observed in these patients.\textsuperscript{10,12} It is plausible that non-heparin triggers of the anti-PF4/heparin immune response, such as glycosaminoglycans released during orthopedic surgery, or bacterial antigens in the case of preceding infection, could account for these unusual clinical observations (bacterial cell walls\textsuperscript{19} and RNA/DNA nucleotides\textsuperscript{20} bind to PF4, recapitulating HIT antigens, and murine\textsuperscript{19} and human bacterial infection\textsuperscript{21} are associated with anti-PF4/heparin antibody formation).

We propose that a definitive diagnosis of spontaneous HIT syndrome should be based upon the following criteria: thrombocytopenia (without alternate explanation), thrombosis, lack of proximate heparin exposure, strong-positive PF4-dependent EIAs (at least two different assays), a strong-positive platelet activation assay (>80% peak serotonin-release) featuring strong heparin-independent platelet activation (>50% serotonin-release at 0 IU/mL heparin), as well as heparin-dependent platelet activation (evident upon serum dilution), and exhibiting also the other characteristic features of HIT sera (inhibition at 100 IU/mL heparin and with Fc receptor-blocking monoclonal antibody). Although fulfilling all of these aforementioned criteria will require serum referral for specialized laboratory investigations (namely, the SRA or another functional assay such as the heparin-induced platelet activation test,\textsuperscript{22} performed using neat and diluted serum and also including buffer control), initial treatment for presumptive spontaneous HIT syndrome need not be delayed; moreover, our proposed rigorous definition will avoid potential over-diagnosis that could occur if a positive EIA alone was used to detect anti-PF4/heparin antibodies in a patient who presented with otherwise unexplained thrombocytopenia.
Acknowledgments

We thank Jo-Ann I. Sheppard for technical assistance and for help in the preparation of the figure. This work was supported by grants T6950 (T.E.W.) from the Heart and Stroke Foundation of Ontario (Toronto, ON).

Authorship

Contribution: T.E.W. designed and supervised the experiments, analyzed the data, interpreted the results, and was the primary author of the paper; P.A.B., J.K., and R.A.B. identified the two patients, referred acute and follow-up patient sera to the McMaster Platelet Immunology Laboratory, and obtained the clinical data. All authors reviewed and approved the final version of the manuscript.

Conflict-of-interest disclosure: T.E.W. has received lecture honoraria from Pfizer Canada and Instrumentation Laboratories, has received research funding from GlaxoSmithKline and Immucor GTI Diagnostics, and has provided expert witness testimony relating to heparin-induced thrombocytopenia. The other authors declare no competing financial interests.

Correspondence: Theodore E. Warkentin, Hamilton Regional Laboratory Medicine Program, Rm 1-180A, Hamilton Health Sciences (General Site), 237 Barton St E, Hamilton, ON L8L2X2, Canada; e-mail: twarken@mcmaster.ca.
References


Table 1. Two patients with spontaneous HIT syndrome.

<table>
<thead>
<tr>
<th>Patient No. (Age, sex)</th>
<th>Day of blood sample</th>
<th>Serotonin-release assay (SRA) % serotonin-release at different UFH concentrations (IU/mL)</th>
<th>PF4-dependent EIAs, Absorbance, OD units (absorbance at high heparin)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>1 (62, M)</td>
<td>Day 0 †</td>
<td>100‡</td>
<td>100‡</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>2 (54, F)</td>
<td>Day 0 †</td>
<td>81§</td>
<td>100§</td>
</tr>
<tr>
<td></td>
<td>Day 28</td>
<td>2</td>
<td>81</td>
</tr>
</tbody>
</table>

Neither patient had laboratory evidence for microangiopathic hemolysis, vasculitis, or antiphospholipid antibodies (negative anticardiolipin and β2-glycoprotein-1 immunoassays, and normal dilute Russell viper venom time).

* For both patients, % serotonin-release was inhibited to 0% at 0.3 IU/mL UFH with Fc receptor-blocking monoclonal antibody (not shown).
† Day 0 represents day of admission, with blood sample obtained before any administration of heparin.
‡ Heparin-dependent platelet activation was evident using 1/4 diluted serum: 37% serotonin-release at 0.1 IU/mL UFH versus 0% release at 0 IU/mL heparin (insufficient serum was available to test at 1/2 diluted serum).
§ Heparin-dependent platelet activation was evident using 1/8 diluted serum (94% serotonin-release at 0.1 IU/mL UFH versus 18% serotonin-release at 0 IU/mL heparin) and using 1/16 diluted serum (85% serotonin-release at 0.1 IU/mL UFH versus 3% release at 0 IU/mL UFH).
** For patient 2, subsequent results in the EIA-IgG/A/M (Immucor GTI) were: 0.95 (day 100) and 0.25 (day 240).
**Figure legend**

Figure 1. Patient 1: Clinical and radiological features. A. Clinical course. B. Thrombotic occlusion at internal carotid artery terminus. C. Flow reestablished to middle cerebral artery (MCA). D. Reocclusion at origin of MCA (note: the unusual problem of five rapid rethromboses may have been related to heparin administration during the mechanical thrombectomies). E. Flow reestablished to MCA and anterior cerebral artery (after infusion of tissue-plasminogen activator). No heparin exposure occurred as a result of the platelet transfusions (platelets were prepared using citrate anticoagulant).

Abbr.: APTT, activated partial thromboplastin time; ASA, acetylsalicylic acid (aspirin); IU, international units; IV, intravenous; SC, subcutaneous; t-PA, tissue plasminogen-activator; U, units; UFH, unfractionated heparin.
Figure 1

A

Serotonin-release assay and enzyme-immunoassays (3 assays):
POSITIVE on day 0 and day 14, see Table

62-yo. male admitted for acute thrombotic stroke
Platelet count = 65 x 10^9/L
No recent hospitalizations, no previous heparin

Platelet transfusions
1U 1U 1U 2U

Platelet count nadir, 27 x 10^9/L

Mechanical thrombectomy
Intra-arterial t-PA (15 mg)
UFH 1000 IU
Complicated by multiple rethromboses
requiring multiple thrombectomies

ASA 325 mg daily x 5

Fondaparinux
7.5 mg SC
daily x 3

Argatroban IV
target 2-times baseline APTT

Warfarin

Days after Admission for Stroke

B
Terminus
Internal carotid artery

C
Middle cerebral artery

D
Reocclusion of middle cerebral artery

E
Anterior cerebral artery
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