Dear Sirs,

Heparin-induced thrombocytopenia (HIT) is a prothrombotic adverse drug effect caused by platelet-activating antibodies that recognise multimolecular complexes of platelet factor 4 (PF4) bound to heparin (1, 2). HIT is almost invariably triggered by proximate treatment with unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH); however, five cases of HIT have been reported in the setting of postoperative thromboprophylaxis with fondaparinux (3–6), a synthetic antithrombin-binding pentasaccharide. Although fondaparinux administered for post-orthopedic surgery thromboprophylaxis is associated with anti-PF4/heparin antibody formation at a frequency similar to that of the LMWH, enoxaparin (7), clinical HIT is rare, probably because antibodies usually do not recognise PF4 in the presence of fondaparinux (7–10).

To date, serum from only one (3) of these five cases of fondaparinux-associated HIT was tested in a platelet activation assay, the serotonin-release assay (SRA). That serum induced strong platelet activation in the absence of pharmacologic heparin, i.e. it had the serological profile of “delayed-onset” (or “autoimmune”) HIT (11). We now report a sixth case of fondaparinux-associated HIT; in contrast to previous cases, this one occurred during medical thromboprophylaxis. The availability of serum from this newly-recognised patient case—and one of the previously reported patients (3)—permits further characterisation of the platelet-activating properties of these antibodies in the presence of heparin or fondaparinux.

A 74-year-old woman with chronic interstitial pulmonary fibrosis, hypertension, and non-insulin-dependent diabetes was admitted to hospital for management of urosepsis; fondaparinux (2.5 mg daily by subcutaneous injections) was started for thromboprophylaxis. The platelet count was 252 x 10^9/l on admission, and initially declined to 139 x 10^9/l before rising to 191 x 10^9/l on day 4 (first day of fondaparinux administration = day 0); during the early in-hospital course, the patient received mechanical ventilation and developed a non-ST-elevation myocardial infarction based upon troponin rise (Fig. 1A). Despite extubation and overall clinical improvement, the patient developed a subsequent 73% decline in platelet count that began on day 5 of fondaparinux prophylaxis, reaching the nadir platelet count of 51 x 10^9/l on day 10. Fondaparinux was discontinued on day 7, and argatroban started the following day. The (pre-argatroban) international normalised ratio (INR) measured 1.2 (normal, 0.9 to 1.1) and the activated-partial thromboplastin time was 31 seconds (s) (normal, 23 to 34 s). The platelet count recovered without clinical or radiological evidence for HIT-associated thrombosis (normal lower-limb venous ultrasound, day 15) or another explanation for thrombocytopenia (i.e. 4Ts score = 6 points) (12). Testing for HIT antibodies was strongly positive (discussed subsequently). At no time was heparin (either UFH or LMWH) administered, including within invasive lines.

A polyclonal PF4-dependent enzyme-immunoassay (EIA) from Gen-Probe GTI Diagnostics (Waukesha, WI, USA) showed strong-positive results (3.00 optical density [OD] units; normal <0.40 units). An “in-house” IgG-specific EIA (McMaster Platelet Immunology Laboratory) yielded strong-positive results (2.75 OD units; normal, <0.45 units). The McMaster SRA showed 80% to 90% serotonin-release in the presence of low-dose heparin (at 0.3 and 0.1 U/ml UFH, respectively) and inhibition (0% release) at 100 U/ml heparin (Fig. 1B; see solid red line); an Fcγ receptor-blocking monoclonal antibody (IV.3) demonstrated that platelet activation occurred through platelet Fcγ receptors (not shown).

Figure 1B also shows results of the SRA performed at serial two-fold dilutions of acute serum from this new patient with putative fondaparinux-associated HIT. Since serum volumes were limited, after the initial diagnostic SRA, subsequent testing was performed using 1/2 and higher dilutions. These studies suggested an increase in percent serotonin-release in the presence of fondaparinux (0.1, 0.4, 0.8, and 1.2 μg/ml); for example, at the 1/8 serum dilution (solid blue line), 55% serotonin-release was observed at 0.1 μg/ml fondaparinux, in comparison with 44% serotonin-release at buffer control.

Figure 1C shows the corresponding results using serum from the previously reported case of fondaparinux-associated HIT (3). This serum also showed evidence for increased platelet activation in the presence of fondaparinux (at 0.1 μg/ml), and was best shown at the 1/128 dilution (green dashes); here the percent serotonin-release increased from 18% to 37%.

For comparison, Figure 1D shows the results from a previously reported case of delayed-onset HIT (patient 1 in [11]). Again, strong heparin-independent platelet activation was found; however, there was no clear increase in platelet activation in the presence of fondaparinux. In contrast, Figure 1E shows the results for thrombocytopenia (i.e. 4Ts score = 6 points) (12). Testing for HIT antibodies was strongly positive (discussed subsequently). At no time was heparin (either UFH or LMWH) administered, including within invasive lines.

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from a previously reported case of severe HIT (patient 11 in [13]) where the platelet count remained low for 13 days post-heparin cessation, and despite treatment with danaparoid and then fondaparinux. This patient’s serum also exhibited strong heparin-independent platelet activation, with some fondaparinux-dependent increase in platelet activation: at 0.1 μg/ml, neat patient serum (solid red line) yielded 71% serotonin-release compared with 49% release at buffer control.

In summary, we report a medical patient who developed clinical and serological evidence of the HIT syndrome beginning approximately one week after receiving fondaparinux thromboprophylaxis. This observation adds to increasing evidence that on rare occasions clinically-evident HIT might result when fondaparinux is administered in a proinflammatory setting, such

Figure 1: Patient with fondaparinux-associated HIT syndrome.
A) Summary of clinical course including serial platelet counts. B-E) Percent serotonin-release is plotted against various concentrations of heparin (0.1, 0.3, 100 U/ml), fondaparinux (0.1, 0.4, 0.8, 1.2, 10, and 100 μg/ml), or buffer control. Results are shown using neat (undiluted) serum as well as serum diluted up to 1/1024 (represented by solid or dashed or dotted lines of different colours – see legend at bottom of figure). For the three patients with evidence of fondaparinux-dependent platelet activation (shown in panels B, C, and E), the actual percent serotonin-release results are indicated in the graph. B) Platelet activation profile of the newly reported case of fondaparinux-associated HIT. The solid red line indicates the results of the initial SRA performed using neat serum. Subsequent studies were performed using 1/2 to 1/16 serum dilutions. C) Serological profile of a previously-reported case of fondaparinux-associated HIT (see text). D) Serological profile of patient with delayed-onset HIT (see text). E) Serological profile of patient with persisting HIT (see text).
as surgery (previous five cases) or a critically-ill medical patient (our new case). In addition, based upon the SRA results for two of these patients with fondaparinux-associated HIT, it is clear that the patient sera exhibit strong heparin-independent platelet activation, i.e. FcγIIa receptor-dependent platelet activation occurring even with buffer control, with inhibition by high concentrations of heparin or fondaparinux characteristic of HIT sera. In both cases, there also is a minor increase in platelet activation in the presence of pharmacological concentrations of fondaparinux. Unfortunately, our studies were limited by severe restriction of patient sample volumes, and thus studies of future patients will be required to determine whether a fondaparinux-dependent increase in platelet activation is a characteristic “marker” of fondaparinux-associated HIT and, indeed, how often this phenomenon is shown by other “strong” HIT sera from patients who develop HIT during treatment with preceding UFH or LMWH.

As proposed elsewhere (10, 14), despite potential immunogenicity of fondaparinux, its relatively low capacity to exacerbate platelet activation by HIT antibodies implies that fondaparinux in therapeutic doses could be an effective treatment for the HIT syndrome, whether triggered by UFH, LMWH, or even by fondaparinux itself.

Conflicts of interest
T.E. Warkentin has received lecture honoraria from Pfizer Canada and Sanofi-Aventis, has provided consulting services to, and/or has received research funding from, Canyon Pharmaceuticals, DiaMed, Gen-Probe GTI Diagnostics, GlaxoSmithKline, and Paringenix, and has provided expert witness testimony relating to HIT. The other authors report no conflicts of interest.

References