

## WHAT IS YOUR CALL?

## Progressive thrombocytopenia after cardiac surgery in a 67-year-old man

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A 67-year-old man who had undergone uncomplicated bioprosthetic replacement of the aortic valve and coronary artery bypass grafting developed transient postoperative thrombocytopenia, followed by a second decline in platelet count. He was clinically stable and did not have new symptoms or postoperative complications. There was no evidence of sepsis, thrombosis or hemorrhage. The patient had received unfractionated heparin (70 000 IU in total) with protamine reversal (600 mg), one unit of pooled platelets and one unit of packed red blood cells during the operation. Enoxaparin (30 mg twice daily) had been given postoperatively for antithrombotic prophylaxis.

The patient's platelet count fell to  $43 \times 10^9/L$  by the second postoperative day and then gradually rose to  $85 \times 10^9/L$  by the fifth day. His platelet count began to decline for a second time on day 6 after surgery and reached  $47 \times 10^9/L$  on day 7, which prompted a consultation with the hematology service (Box 1). A complete blood count showed that the patient had mild, stable, postoperative anemia (hemoglobin level 82 g/L and mean corpuscular volume 95 fL) and a normal leukocyte count ( $6.2 \times 10^9/L$ ) with a normal differential. A peripheral blood film confirmed thrombocytopenia, with no morphologic abnormalities in the erythrocytes or leukocytes. Prothrombin and activated partial thromboplastin times were within normal limits. The patient's lactate dehydrogenase level was 207 (normal 125–220) U/L.

### What is the most likely diagnosis at the seventh postoperative day?

- a. Dilutional thrombocytopenia
- b. Heparin-induced thrombocytopenia
- c. Post-transfusion purpura
- d. Thrombotic thrombocytopenic purpura

A tentative diagnosis of heparin-induced thrombocytopenia, an immune-mediated thrombocytopenia

resulting in platelet activation and hypercoagulability, was made (b). Dilutional thrombocytopenia could explain the initial decline in platelet count, which reached its nadir on the second postoperative day, and the subsequent recovery of the platelet count to  $85 \times 10^9/L$ . The nadir of postoperative thrombocytopenia can occur anytime between days 1 and 4 after surgery (most commonly occurring on day 2). Once the platelet count begins to rise, the ultimate peak count occurs on about day 14, with a gradual restoration to preoperative levels over the following one to two weeks.<sup>1</sup> In contrast, new-onset thrombocytopenia on or after postoperative day 5, which was observed in our patient, is not consistent with a diagnosis of dilutional thrombocytopenia.

Post-transfusion purpura is a rare disorder that occurs almost exclusively in women with a remote history of pregnancy or transfusion; less than 5% of cases are seen in men who have had previous blood transfusion.<sup>2</sup> Post-transfusion purpura presents with severe thrombocytopenia (platelet count  $< 10 \times 10^9/L$ ) and mucocutaneous bleeding that occurs 5–10 days after transfusion with a platelet-containing product (usually packed red blood cells).<sup>2</sup>

#### Competing interests:

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#### Box 1: Sequence of platelet counts in a 67-year-old man who underwent cardiac surgery\*

- Preoperative: 158
- Intraoperative: 71
- Postoperative
  - Immediate: 103†
  - Day 1: 86
  - Day 2: 43
  - Day 3: 61
  - Day 4: 78
  - Day 5: 85
  - Day 6: 73
  - Day 7: 47

\*All platelet counts are  $\times 10^9/L$  (normal range 150–400).

†Patient received an intraoperative platelet transfusion.

Thrombotic thrombocytopenic purpura can occur postoperatively, in particular following cardiac surgery,<sup>3</sup> but it is rare. The absence of schistocytes on the blood film and a normal lactate dehydrogenase level ruled out this diagnosis in our patient.

A presumptive diagnosis of heparin-induced thrombocytopenia was made, and a therapeutic dose of fondaparinux (7.5 mg/d) was started based on expert opinion.<sup>4</sup> A polyspecific enzyme-linked immunosorbent assay (ELISA) for antiplatelet factor 4/heparin antibodies was strongly positive (optical density 2.5 [normal < 0.40]). Bilateral Doppler and compression ultrasound scans of the legs were obtained to rule out subclinical thrombosis,<sup>3</sup> because its presence would influence the required duration of anticoagulation. The ultrasound images showed no evidence of deep vein thrombosis, and there were no clinical signs or symptoms of other venous or arterial thromboembolism.

Despite the change to treatment with fondaparinux, the patient's platelet count continued to decline over the next four days, from  $47 \times 10^9/\text{L}$  on postoperative day 7 to  $13 \times 10^9/\text{L}$  on day 11. All other parameters of the complete blood count and peripheral blood film were unchanged. Prothrombin and activated thromboplastin times remained within normal limits, and the fibrinogen level was elevated (4.5 [normal 1.5–3.5] g/L). The hematology service was asked to reevaluate therapy.

### What is the most appropriate intervention for this patient?

- Stop treatment with fondaparinux and start high-dose intravenous immunoglobulin treatment
- Stop treatment with fondaparinux and start treatment with a direct thrombin inhibitor
- Continue treatment with fondaparinux
- Stop treatment with fondaparinux without starting treatment with a different anticoagulant

Fondaparinux treatment was continued (c) for a presumptive diagnosis of heparin-induced thrombocytopenia, based on the presence of antibodies characteristic of the “delayed-onset” type. High-dose intravenous immunoglobulin treatment would be an appropriate treatment for post-transfusion purpura. A direct thrombin inhibitor, such as argatroban or bivalirudin, would be an appropriate treatment for heparin-induced thrombocytopenia, in keeping with recent evidence-based guidelines;<sup>6</sup> however, titration of the dose of these agents using the laboratory coagulation

parameters can be confounded by concomitant disseminated intravascular coagulation,<sup>7</sup> a complication occurring in up to 25% of patients with delayed-onset heparin-induced thrombocytopenia.<sup>8,9</sup> A case series of patients with well-documented heparin-induced thrombocytopenia reported that fondaparinux was a safe and effective treatment for this condition.<sup>10</sup> The occurrence or perpetuation of heparin-induced thrombocytopenia in patients taking fondaparinux is rare.<sup>11</sup> Most importantly, the usual progression of the delayed-onset variant of heparin-induced thrombocytopenia is characterized by progressive thrombocytopenia that reaches its nadir between days 10 and 17 after surgery.<sup>8,9</sup>

Given that our patient was otherwise doing well, without clinical evidence of thrombosis or overt disseminated intravascular coagulation, the decision was made to continue treatment with fondaparinux. Discontinuation of fondaparinux without starting treatment with a different anticoagulant would put the patient at risk of venous or arterial thromboembolism, which occurs in over 50% of patients with heparin-induced thrombocytopenia if adequate anticoagulation is not provided.<sup>12</sup> Current guidelines and an expert review recommend a minimum of four weeks of therapeutic treatment with a non-heparin anticoagulant in patients who have heparin-induced thrombocytopenia without thrombosis.<sup>5,6</sup>

### Which result of a serotonin release assay would confirm the diagnosis?

- Serotonin release at a dose of 0.1 IU/mL heparin, but not at a dose of 0 or 100 IU/mL heparin
- Serotonin release at doses of 0 and 0.1 IU/mL heparin, but not at a dose of 100 IU/mL heparin
- No serotonin release at any concentration of heparin
- Serotonin release only with doses of fondaparinux

The serotonin release assay is a diagnostic test for heparin-induced thrombocytopenia that measures the ability of the antiplatelet factor 4/heparin immunoglobulin G (IgG) antibodies to activate platelets and induce serotonin release in a sample of serum. In our patient, the results of the assay confirmed strong reactivity (b) both in the presence of heparin at a therapeutic dose (day 11 serum sample: peak 92% serotonin release at 0.1 IU/mL unfractionated heparin) and in the absence of heparin (peak 81% serotonin release at 0 IU/mL unfractionated heparin)

(Appendix 1, available at [www.cmaj.ca/lookup/suppl/doi:10.1503/cmaj.131449/-/DC1](http://www.cmaj.ca/lookup/suppl/doi:10.1503/cmaj.131449/-/DC1)). Platelet activation was inhibited at a high concentration of heparin (0% serotonin release at a dose of 100 IU/mL unfractionated heparin). The strong serum-induced activation of platelets observed in the absence of heparin (buffer control) is an important serologic feature of delayed-onset heparin-induced thrombocytopenia.<sup>9</sup>

Serotonin release at a dose of 0.1 IU/mL heparin, but not a dose of 0 or 100 IU/mL heparin, would be characteristic of most patients with heparin-induced thrombocytopenia, in whom strong reactivity in the serotonin release assay is observed only when the patient is receiving therapeutic concentrations of heparin. No serotonin release at any concentration of heparin argues against a diagnosis of heparin-induced thrombocytopenia.

Serotonin release only with doses of fondaparinux would implicate fondaparinux as a causal agent of the thrombocytopenia and would suggest that this agent was provoking platelet activation and an associated prothrombotic state. There was no increase in reactivity over the buffer control at any dose of fondaparinux tested; this included tests of patient serum diluted 1:16 and 1:64, in which less than 5% reactivity was observed at a dose of 0 IU/mL heparin and at all concentrations of fondaparinux, but strong heparin-dependent activation of platelets (> 80% serotonin release) remained (Appendix 1). Because the addition of fondaparinux did not increase the degree of serotonin release compared with that observed with the buffer control, fondaparinux cross-reactivity was ruled out.

The patient continued to receive therapeutic anticoagulation (which was changed to treatment with rivaroxaban upon discharge because of patient preference). The gradual elevation of his platelet count corresponded to an observed progressive decline in serum-induced activation of platelets in the absence of heparin.

## Discussion

Heparin-induced thrombocytopenia is a well-characterized, prothrombotic disorder caused by platelet-activating IgG antibodies that recognize multimolecular complexes of platelet factor 4 (a cationic protein) bound to anionic heparin through charge–charge interactions.<sup>8</sup> Platelet activation occurs when the platelet factor 4/heparin/IgG complexes form in situ on the surface of platelets, cross-linking the platelet FcγIIa receptors, thereby producing strong platelet activation in association with a marked procoagulant response.

Clinically, heparin-induced thrombocytopenia typically presents 5–10 days after an immunizing exposure to heparin, which is usually heparin given intraoperatively (e.g., during cardiac or vascular surgery) or soon after surgery (e.g., as usual postoperative thromboprophylaxis).<sup>12</sup> The common occurrence of heparin-induced thrombocytopenia postoperatively is believed to result from the combination of surgery (with the release of platelet factor 4 from activated platelets) and the administration of heparin during or after surgery, triggering the formation of the immunogenic platelet factor 4/heparin complexes.<sup>13</sup>

In our patient, the initial picture of a second decline in the platelet count that began on the fifth postoperative day and while the patient was receiving low-molecular-weight heparin was consistent with heparin-induced thrombocytopenia. However, as the case evolved, several points seemed to argue against the diagnosis, in particular, the progressive decline of the patient's platelet count for nearly a week following the cessation of heparin treatment. One possible explanation for this was concomitant disseminated intravascular coagulation, a well-described complication of heparin-induced thrombocytopenia.<sup>8,9</sup> However, disseminated intravascular coagulation is unlikely to progress if the patient is receiving effective anticoagulation; our patient was receiving fondaparinux at a therapeutic dose. In addition, stable fibrinogen levels and coagulation parameters (activated partial thromboplastin time and prothrombin time/international normalized ratio) within the normal range suggested that there was no progression to disseminated intravascular coagulation.

It is commonly thought that severe thrombocytopenia (platelet count < 20 × 10<sup>9</sup>/L) argues against a diagnosis of heparin-induced thrombocytopenia; however, it occurs in 5%–10% of patients with heparin-induced thrombocytopenia and in about 50% of patients with delayed-onset heparin-induced thrombocytopenia.<sup>9</sup> Such patients are at increased risk of disseminated intravascular coagulation and microthrombosis associated with heparin-induced thrombocytopenia. The lowest recorded nadir for a platelet count in a patient with serologically confirmed heparin-induced thrombocytopenia is 2 × 10<sup>9</sup>/L.<sup>8</sup>

## Delayed-onset heparin-induced thrombocytopenia

Delayed-onset heparin-induced thrombocytopenia was initially reported in a case series of 12 patients in whom heparin-induced thrombocytopenia began five or more days after their last exposure to heparin.<sup>9</sup> Recently, the term “delayed-onset heparin-induced thrombocytopenia” also

has been used to describe heparin-induced thrombocytopenia that worsens after heparin treatment ends,<sup>8</sup> which occurred in our case. Our patient's platelet count recovered relatively slowly; this is consistent with the finding that the normalization of the platelet count may follow a protracted course in patients with delayed-onset heparin-induced thrombocytopenia and associated strong reactivity in the absence of heparin in the serotonin release assay.<sup>9</sup> An evaluation on day 44 after surgery showed that the serum of our patient remained strongly positive in the serotonin release assay at therapeutic heparin doses (Appendix 1). In addition, whereas the reactivity of the serotonin release assay in the absence of heparin declined over time (with a corresponding gradual increase in the patient's platelet count to  $61 \times 10^9/L$ ), there was still some residual reactivity (10% serotonin release), which explains the persisting degree of thrombocytopenia in the patient. At day 88 after surgery, the platelet count was  $111 \times 10^9/L$ , and there was essentially no reactivity in the serotonin release assay in the absence of heparin (3% serotonin release).

### Diagnostic testing for heparin-induced thrombocytopenia

The need to make a timely diagnosis and start appropriate therapy requires an awareness of how heparin-induced thrombocytopenia can present and progress in a patient, because it often takes several days to confirm the diagnosis with laboratory tests. However, these tests are critical and can inform the clinician's understanding and characterization of the disease process.

Enzyme-linked immunosorbent assay is undertaken initially and identifies the presence of high levels of antiplatelet factor 4/heparin antibodies in patient serum; its high sensitivity (about 99%)<sup>14,15</sup> allows heparin-induced thrombocytopenia to be ruled out if the result is negative, whereas the strength of a positive result can predict the likelihood of true heparin-induced thrombocytopenia. As in our patient, higher optical density levels ( $> 2.00$ ) have been strongly associated with the presence of heparin-dependent, platelet-activating IgG antibodies, which are implicated in heparin-induced thrombocytopenia: up to 90% of patients with optical density levels greater than 2.0 in the ELISA will have a positive result in the serotonin release assay.<sup>16,17</sup> When the optical density is not considered, the specificity of a positive ELISA result is limited and has been estimated to be as low as 26%.<sup>18</sup>

As described earlier, the presence of antiplatelet factor 4/heparin antibodies in a patient's serum is not sufficient for a diagnosis of heparin-induced thrombocytopenia. In fact, the

serum of most patients with such antibodies will not be able to activate platelets in a serotonin release assay, which indicates that these antibodies are not pathogenic. Conversely, in most true cases of heparin-induced thrombocytopenia, the serotonin release assay will demonstrate the ability of the patient's serum to induce platelet activation only at therapeutic concentrations of heparin, with minor or no platelet activation in the absence of heparin, and an inhibition of platelet activation at very high concentrations of heparin.<sup>9</sup> Currently, the serotonin release assay is performed in only one laboratory in Canada (McMaster Platelet Immunology Laboratory, Hamilton, Ont.).

The serotonin release assay provides three important contributions to our case. First, the test is more specific for heparin-induced thrombocytopenia than the ELISA and therefore strongly suggests a diagnosis of heparin-induced thrombocytopenia, despite our patient's atypical clinical features. Second, the strong, serum-induced platelet activation at a concentration of 0 IU/mL heparin is characteristic of delayed-onset heparin-induced thrombocytopenia, which helps to explain both the dramatic progression of thrombocytopenia in the absence of further treatment with heparin and the subsequent slow recovery of the platelet count in our patient. The sera of patients with delayed-onset heparin-induced thrombocytopenia, when compared with the sera of patients with usual heparin-induced thrombocytopenia, have strong serotonin release ( $\geq 50\%$ , and often  $> 80\%$ ) both in the presence and in the absence of therapeutic concentrations of heparin. Serotonin release will be inhibited at a dose of 100 IU/mL heparin in all sera of patients with heparin-induced thrombocytopenia.<sup>9</sup> Finally, the serotonin release assay ruled out fondaparinux cross-reactivity as the cause of the progressive decline in the platelet count in our patient while he received this drug.

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