The complex diagnosis of post-transfusion purpura: a case report

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SUMMARY

Post-transfusion purpura (PTP) is a rare but potential lethal transfusion complication. This immune-mediated transfusion reaction can occur three to ten days after a transfusion of a product containing platelets or platelet antigens. Antibodies against human platelet antigen (HPA)-1a are most frequently identified as causal, although antibodies against other HPA antigens have been demonstrated. The rarity of PTP and its manifestation under complex clinical conditions associated with thrombocytopenia often delays the clinical and laboratory suspicion to include PTP in the differential diagnosis. The treatment mainly consists of suppression of the platelet destruction. We present a patient illustrating the typical complexity of the clinical manifestation, diagnosis and treatment of PTP.

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INTRODUCTION

Post-transfusion purpura (PTP) is a rare immune-mediated transfusion complication with an estimated incidence of 1:50,000 to 1:100,000 blood transfusions and a higher incidence in women (female-to-male ratio 5:1). A PTP usually develops three to ten days after transfusion with platelet or platelet-antigen containing products. Sometimes a febrile reaction occurred at the time of the transfusion. As a result of antibodies formed against the transfused platelets, destruction and removal from the circulation of the transfused and the patient's own platelets takes place. PTP often results in very deep thrombocytopenia that can be accompanied by both intracranial and gastrointestinal bleeding.[1] The destruction of the transfused donor platelets is explained by the formation of alloantibodies against human platelet antigen (HPA), often against the high-frequency HPA-1a antigen present in the previously transfused products and absent in the patient and absent in 2% of the population. Generally speaking, the formation of these alloantibodies involves a 'booster' response because the patient has already been sensitised to the relevant antigen during pregnancy or transfusion. The most frequently occurring alloantibody is HPA-1a, but antibodies against almost all other HPA antigens have been described.[2] There are several theories about the cause for the breakdown of the patient's own platelets, which are negative for the antigen against which the alloantibodies are targeted. The platelets may absorb HPA antigen-antibody complexes and are subsequently removed as 'innocent bystanders'.[3] A second, more probable theory is that the production of autoantibodies, in addition to the specific allo-antibodies, against non-polymorphic glycoprotein structures on platelets is associated with the strong 'booster' response.[4] HPA-incompatible platelets are removed from the circulation by the antibodies and can further stimulate the formation of antibodies. HPA antigen-compatible platelets are destroyed at the same rate as the patient's own platelets.

A rapid diagnosis is important to prevent unnecessary platelet transfusions, which can aggravate haemorrhagic complications. Good differential diagnosis can lead to targeted and rapid laboratory testing to determine adequate treatment.

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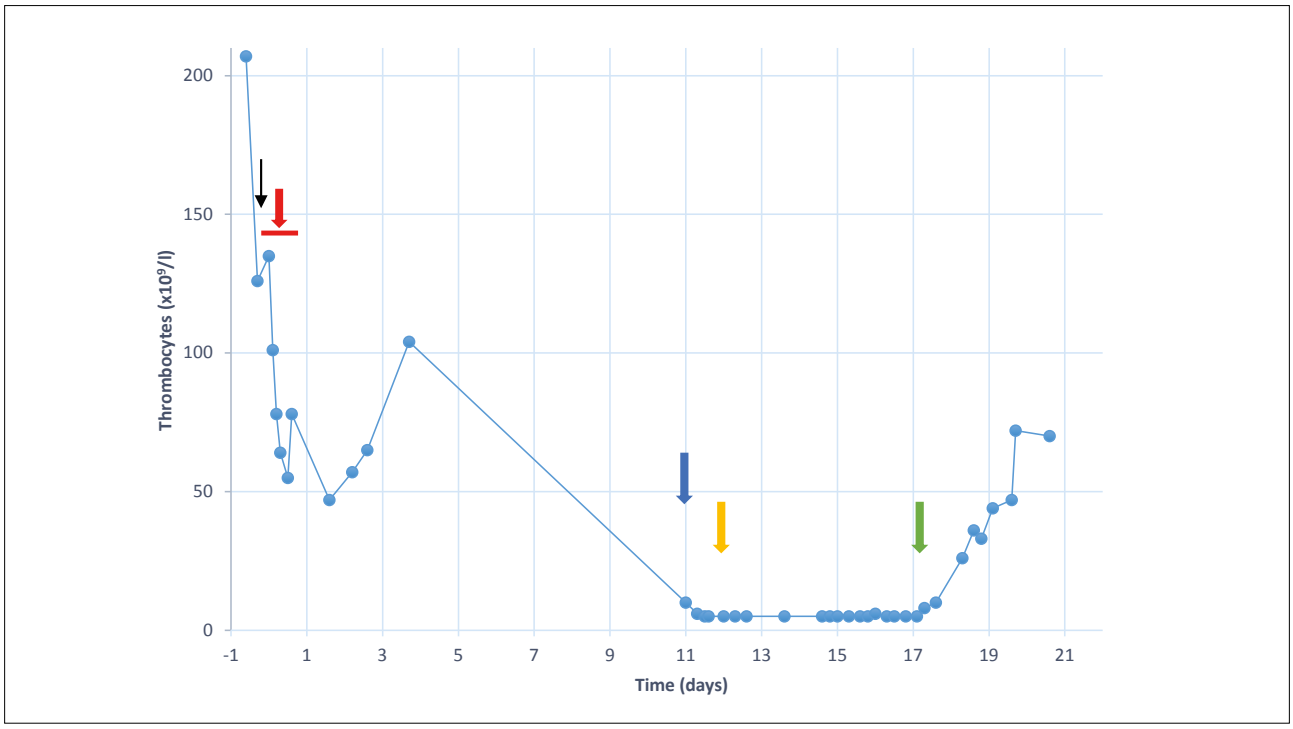

FIGURE 1. Platelet concentration course. Time zero corresponds to the aortic ascending replacement and is indicated by the black arrow. The red line and arrow indicate the time in the operating theatre, where a total of thirteen erythrocyte concentrates, twelve units of fresh frozen plasma and six platelet concentrates have been administered. The blue arrow indicates the resternotomy in which four human platelet antigen-1a negative 'pedipack' (single buffy-coat derived) platelets, seven erythrocyte concentrates and four units of fresh frozen plasma are administered. The orange arrow corresponds to the start of intravenous immunoglobulin and prednisone. The green arrow indicates the second intravenous immunoglobulin dose.

The fact that PTP usually manifests itself under complex clinical conditions is illustrated by the following case study of a patient with a complex clinical presentation, diagnostics, product selection and treatment.

CASE REPORT

A 57-year-old woman (two children) presents herself in the emergency room of a referring hospital with a haemorrhagic diathesis and thrombocytopenia (<5 x 10^9/l) and renal dysfunction. Petechiae are observed all over the body and there is anaemia and melaena. Clinically, and confirmed with echocardiography, there is a cardiac tamponade and pericardial fluid. Two weeks earlier, the patient had an aortic ascending replacement due to a type A dissection. There was a normal blood count before surgery. During this procedure, the patient received thirteen erythrocyte concentrates, twelve units of fresh frozen plasma and six platelet concentrates. When presented at the emergency room, the patient receives an erythrocyte concentrate and two platelet concentrates, without yield. The (further) course of the platelet concentration is shown in *Figure 1*. Diagnostics are used to investigate the cause of the thrombocytopenia in combination with the absence of a platelet transfusion yield (*Table 1*). Normal erythrocyte morphology and absent fragmentocytes are initially observed, and pseudothrombopaenia is excluded. Although such a deep thrombocytopenia is unusual, heparin-induced thrombocytopenia (HIT) is excluded by means of a heparin platelet factor 4 enzyme-linked immunosorbent assay (ELISA). Medication-induced thrombocytopenia, other than heparin, cannot be completely excluded. There are no abnormalities in coagulation that are compatible with diffuse intravascular coagulation. Normal ADAMTS13 activity and absent fragmentocytes make thrombotic thrombocytopenic purpura and haemolytic-uremic syndrome unlikely. Only after these causes of thrombocytopenia have been excluded, the rare entity of a PTP is considered. In connection with the suspicion of PTP, usually caused by HPA-1a antibodies, the referring hospital orders four HPA-1a negative 'pedipack' platelet concentrates. During the admission, the patient deteriorates hemodynamically and is transferred to our hospital. A resternotomy is performed upon arrival. A large amount of pericardial and pleural fluid is drained. A temporary epicardial ventricular pacemaker wire is also inserted. During this procedure, the four HPA-1a negative 'pedipack' platelet

TABLE 1. Differential diagnosis in thrombocytopenia (after blood transfusion) and associated diagnostics.

DD	Diagnostics	Case results
Pseudothrombopaenia	Platelet count in EDTA blood Platelet count in citrate blood	Absence of platelet aggregates
TMA	Fragmentocytes in the blood count ADAMTS13 activity	Absent 69%
ITP	Diagnosis per exclusionem	
DIC	APTT PT Fibrinogen D-dimers	25 sec (ref 25-35 sec) 19.3 sec (ref 25.5-14.5 sec) 3.3 g/l (ref 2.0-4.0 g/l) not implemented
Medications	Discontinue recent/suspicious medication	No action has been taken on medication use as a possible cause of the thrombopaenia
HIT	Platelets >15 x 10^9/l Antibodies against the PF4 heparin complex by means of ELISA	10 x 10^9/l (ref 150-400 10^9/l) negative
PTP	Antibodies against a HPA-Ag absent in patient in MAIPA and/or PIFT using HPA-typed platelets HPA genotyping of the patient	Different antibodies against both HPA and HLA antibodies are identified (*Table 2*)
Passive HPA Ab	Antibodies against polymorphic HPA-Ag present in the donor	Not further investigated

TMA: thrombotic microangiopathy, ITP: immune thrombocytopenic purpura, DIC: diffuse intravascular coagulation, HIT: heparin-induced thrombocytopenia, PTP: post-transfusion purpura, HPA: human platelet antigen(s), Ab: antibody, PT: prothrombin time, APTT: activated partial thromboplastin time, PF4: platelet factor 4, ELISA: enzyme-linked immunosorbent assay, Ag: antigen, MAIPA: monoclonal antibody immobilisation of platelet antigen, PIFT: platelet immunofluorescence technique.

concentrates are administered without increment. A high dose of intravenous immunoglobulin (IVIG; 1 g/kg) and prednisone (1 mg/kg) is also started to reduce platelet destruction and to suppress the immune system.[7]

Further research into PTP is being done by Sanquin Diagnostics in Amsterdam. The presence of HPA antibodies is investigated using monoclonal antibody immobilization of platelet antigens (MAIPA) and platelet immunofluorescence technique (PIFT). A panel with HPA-1, -2, -3, -5 and -15-typed donor platelet suspensions is used for this. The patient's HPA genotyping is also performed and research is conducted into the presence of HLA class I antibodies using the Luminex beads screening and single antigen technique. The investigation takes three days. Pending the diagnosis, a (provisional) policy is formulated to be cautious with prophylactic and therapeutic platelet transfusions and to maintain Hb above 6.5 mmol/l, with the aim of reducing bleeding tendencies.[10]

Strong alloantibodies against HPA-5a are detected in the patient together with weak autoantibodies against glycoprotein (GP) Ia/IIa, on which HPA-5 is located (*Figure 2* & *Table 2*). In addition, weak reactive antibodies are found against αIIbβ3, while it remains unclear whether this concerns allo- or autoantibodies. The reaction pattern suggests that antibodies are present against HPA-1b and -3a located on this glycoprotein. Despite the use of specific extensive typed donor platelet panels, the presence of the latter antibodies cannot, however, be fully proven. The patient's complete HPA genotyping and the antibodies against HPA antigens are listed in *Table 2*. The study confirms the diagnosis of PTP in an HPA-5a negative patient and strong anti-HPA-5a antibodies after recent multiple transfusions. The patient also possesses multiple HLA class I antibodies (*Table 3*), possibly due to pregnancies and recent transfusions.

TABLE 2. Patient human platelet antigen/antibody.

Name	Allele frequency Caucasians	Antigen	Antibody in the case patient
HPA-1a	98%	Present	.
HPA-1b	28%	Absent	Weak αIIbβ3 antibodies*
HPA-2a	99%	Present	.
HPA-2b	16%	Present	.
HPA-3a	84%	Absent	Weak αIIbβ3 antibodies*
HPA-3b	63%	Present	.
HPA-5a	99%	Absent	Present + anti-GPIa/IIa antibody**
HPA-5b	15%	Present	Antibody against GPIa/IIa**
HPA-15a	74%	Present	.
HPA-15b	76%	Absent	.

HPA: human platelet antigen,
**located on this glycoprotein HPA-1b and -3a,*
***HPA-5 is localised on this glycoprotein.*

In connection with possible elective procedures such as removal of the drain, removal of the temporary pacemaker and other possible complications, Sanquin Blood Supply is asked to search for the most compatible HPA and human leukocyte antigen (HLA)-typed donors. There are no donors compatible for both HLA and HPA antibodies. It is decided to call the three HPA-1b, -3a and -5a negative donors, so that HPA-compatible concentrates are present for administration in case of complications. It is agreed with the clinic that an HPA-compatible platelet concentrate is given in case of removal of the drain and in the event of an imminent infection. In the event of a bleeding, two HPA-compatible platelet concentrates will be given, but if possible, natural recovery should be awaited.

Because the platelet count remains low, on day seven of admission, it is decided to administer a second IVIG dose (1g/kg). On day eight of admission, the patient has a platelet count of 10 x 10^9/l. Given the risk of infection, it is decided to remove the pericardial drain. Platelet concentrates are not administered, and the procedure runs without complications. The platelet count rose to 95 x 10^9/l on the 12th day after resternotomy. The epicardial pacemaker wire is removed without complications. The patient is returned to the referring hospital. There, the patient fully recovers.

DISCUSSION

The patient in this case presents with a severe thrombocytopenia and haemorrhagic diathesis that is refractory to platelet transfusions and originated after transfusion of multiple blood products, administered during the aortic ascending replacement two weeks before. Apart from the severe thrombocytopenia, intervention is needed for the pericardial tamponade. It is only after the exclusion of various other causes for thrombocytopenia that PTP is diagnosed, a rare but potentially life-threatening transfusion complication. About 300 cases have been described in the literature since 1961.[5] Most patients recover after 1-4 weeks, but 15% has a fatal outcome.

The vast majority of PTP case is associated with a strong 'booster' response to HPA-1a. Although only 2% of the Caucasian population is negative for HPA-1a, immunisation against HPA-1a occurs in approximately 1:500 pregnancies.[6] Occasionally, antibodies against HPA-5b, HPA-1b, HPA-3a, HPA-3b, HPA-4a and HPA-5a are identified in PTP.[4,7-9]

In this case, on suspicion of PTP, pending the results of the laboratory tests, the administration of HPA-1a negative platelets was chosen, without result. Ultimately, strong alloantibodies against HPA-5a (located on the GPIa/IIa complex) in combination with weaker antibodies against this platelet

TABLE 3. Patient human leukocyte antigen antibodies.

Class	Antibody against
HLA-class I	HLA-A2
	HLA-A11
	HLA-A30(19)
	HLA-A74(19)
	HLA-B52(5)
	HLA-B44(12)
	HLA-B45(12)
	HLA-B62(15)
	HLA-B18

HLA: human leukocyte antigen.

TABLE 4. Blood group genotyping of patient.

Name	Antibody	Antigen
C	.	Absent
C (small)	.	Present
E	.	Absent
E (small)	.	Present
K	.	Absent
K (small)	.	Present
Fy(a)	.	Present
Fy(b)	.	Present
Jk(a)	.	Present
Jk(b)	.	Absent
S	.	Present
S (small)	.	Present

membrane complex could be identified. The biallelic HPA-5 system located on the GPIa/IIa complex has a relatively low expression at approximately 2,000 copies per platelet and is involved in, among other functions, binding to collagen. HPA-5a is a high frequency antigen (>99.9%), and therefore, immunisation against this antigen is rarely seen.

This case was further complicated by the presence of HLA class I antibodies and the antibodies against αIIbβ3, which possibly also reduced the increment after platelet transfusions.

Despite a single case in which HLA class I antibodies are associated with a PTP image, this relationship is unlikely.[10] The weak reactive antibodies to GPIa/IIa and αIIbβ3 may be an expression of the strong and broad immune response that characterises PTP. A similar case to our patient was previously described.[9]

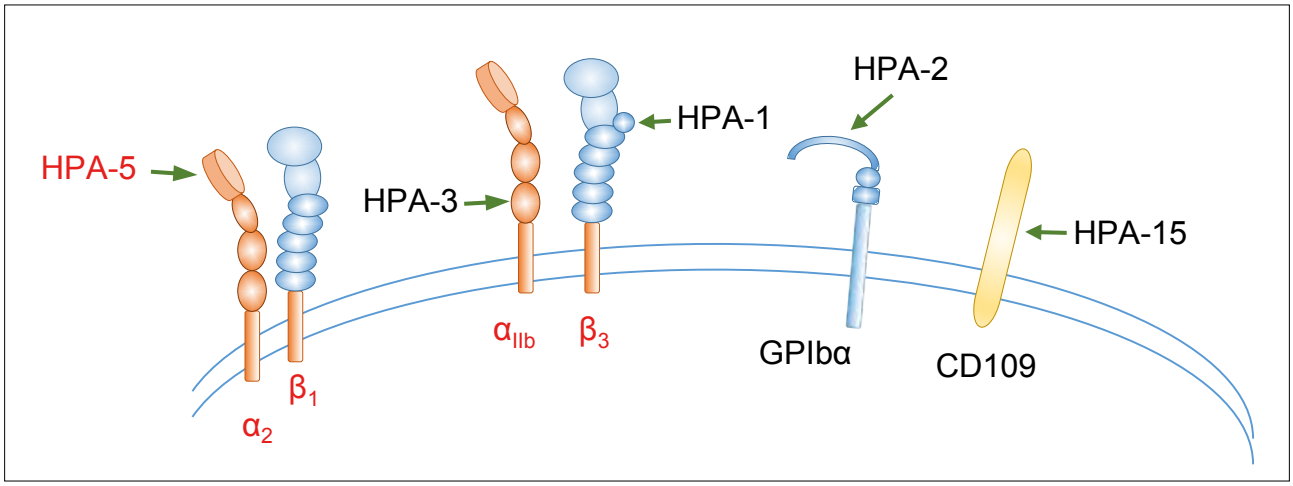

FIGURE 2. Human platelet antigens on the platelets. A schematic representation of various human platelet antigens as part of different glycoprotein on the platelet membrane. The human platelet antigens and glycoprotein structures against which antibodies have been found are marked in red.

KEY MESSAGES FOR CLINICAL PRACTICE

1. **In the case of a clinical presentation of severe thrombocytopenia, it is necessary to check whether a transfusion has taken place in the last two weeks, regardless of the type of product.**
2. **On average, a heparin-induced thrombocytopenia presents with a platelet count >20 x 10^9/l, and post-transfusion purpura usually has platelet counts of <15 x 10^9/l. A rapidly executable heparin platelet factor 4 enzyme-linked immunosorbent assay can, if negative, help exclude heparin-induced thrombocytopenia.**
3. **Given the low frequency of post-transfusion purpura there is no evidence-based treatment available and the treatment is therefore based on expert opinion and literature.**
4. **Intravenous immunoglobulin often in combination with prednisone, is given to suppress the immune system and to inhibit antibody-mediated platelet destruction. An increase in platelets can be expected four days after starting this therapy. If no increase in platelet count has occurred after four days, repeating the treatment with intravenous immunoglobulin may be considered.**
5. **Prophylactic platelet transfusions are discouraged. In case of severe bleeding, a platelet transfusion (preferably HPA-compatible) may be considered. To improve the platelet function, it is possible to aim for an Hb >6.5 mmol/l.**

In the end, the patient was fully recovered. If the patient needs a platelet transfusion in the future, it seems safe to select a platelet concentrate that is as compatible as possible, at least HPA-5a negative, in order not to 'booster' HPA antibodies again. Given the HLA antibodies present, finding fully compatible platelets will be problematic. In case of active and random bleeding, available platelet concentrates have to be administered and it is advised to consider simultaneous treatment with IVIG and prednisone. However, these recommendations are not based on evidence. This patient presented with a very challenging combination of platelet antibodies. It is therefore remarkable that no erythrocyte antibodies were found, given the activated immune system and the 13 transfused erythrocyte concentrates. Extensive blood group genotyping on the erythrocytes showed that the patient, blood type B Rhesus-D negative, was heterozygous for almost all clinically relevant blood groups and could only make an anti-Jk(b) (*Table 4*).

The low frequency of PTP means that both the clinicians and the clinical chemistry laboratories are not easily aware of this condition. The diagnosis for PTP takes time and the treatment consists mainly of immune suppression. In our experience, communication between the various clinical and laboratory specialisms for the preparation of an anticipatory policy plan is of great importance to guarantee care for these complex patients.

REFERENCES

1. Lubenow N, Eichler P, Albrecht D, et al. Very low platelet counts in post-transfusion purpura falsely diagnosed as heparin-induced thrombocytopenia: Report of four cases and review of literature. Thromb Res. 2000;100(3):115-25.
2. Kaplan C. Post-transfusion purpura. TATM. 2002;4(2):26-8.
3. Dieleman LA, Brand A, Claas FH, et al. Acquired Zwa antigen on Zwa negative platelets demonstrated by Western blotting. Br J Haematol. 1989;72(4):539-42.
4. Taaning E, Tønnesen F, et al. Pan-reactive platelet antibodies in post-transfusion purpura. Vox Sang. 1999;76(2):120-3.
5. Hendrickson JE, Hillyer CD. Noninfectious serious hazards of transfusion. Anesth Analg. 2009;108(3):759-69.
6. Williamson LM, Stainsby D, Jones H, et al. The impact of universal leukodepletion of the blood supply on hemovigilance reports of posttransfusion purpura and transfusion-associated graft-versus-host disease. Transfusion. 2007;47(8):1455-67.
7. Hayashi T, Hirayama F, et al. Advances in alloimmune thrombocytopenia: perspectives on current concepts of human platelet antigens, antibody detection strategies, and genotyping. Blood Transfus. 2015;13(3):380-90.
8. Barba P, Pallarés P, Nogués N, et al. Post-transfusion purpura caused by anti-HPA-3a antibodies that are only detectable using whole platelets in the platelet immunofluorescence test. Transfus Med. 2010;20(3):200-2.
9. Ziman A, Klapper E, Pepkowitz S, et al. A second case of post-transfusion purpura caused by HPA-5a antibodies: successful treatment with intravenous immunoglobulin. Vox Sang. 2002;83(2):165-6.
10. Arewa OP, Nahirniak S, Clarke G, et al. Anti-HPA-1b mediated posttransfusion purpura: a case report. Case Rep Med. 2013;2013:568364.