

Case Reports

Platelet Refractoriness in a Pediatric ECMO Patient: A Case Report

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Abstract: Thrombocytopenia is a life-threatening condition, the severity of which is exacerbated further if the patient requires anticoagulation and is refractory to platelet transfusion. This is the first report of an infant undergoing extracorporeal support with immune-mediated platelet refractoriness. A 19-month-old girl, with a complex cardiac history, required extracorporeal support because of deterioration 8 days post-cardiac surgery. The child suffered from ongoing thrombocytopenia, unresponsive to multiple platelet transfusions. An incremental rise in the platelet count was achieved following transfusion of human leukocyte antigen–matched platelets, although this was unsuccessful with

subsequent transfusions of matched platelets. Following 7 days on extracorporeal membrane oxygenation (ECMO), without cardiac improvement and likely poor prognosis, treatment was withdrawn and the patient died. The management of immune-mediated platelet refractoriness, in an anticoagulated patient on ECMO, requires early diagnosis and timely intervention to achieve a good outcome for the child. An understanding of the condition and a multidisciplinary approach to its treatment will assist in effective direction of medical therapy. **Keywords:** ECMO, platelet refractoriness, transfusion, pediatric. *J Extra Corpor Technol. 2019; 51:83–7*

Pediatric cardiac surgery and pediatric extracorporeal membrane oxygenation (ECMO) can result in varying degrees of challenge to the hemostatic system. Platelets are a key component of the hemostatic system, and vital in the maintenance of vascular integrity.

The platelet is an anucleate discoid-shaped cell, which is three to five microns in diameter and .5 microns in depth. The average circulating platelet count is $150\text{--}400 \times 10^9/\text{L}$. Where damage to the integrity of the vascular system occurs, the platelets will adhere to this site, form a plug, provide a scaffold, and secrete cytokines that are required for vascular repair (1).

Thrombocytopenia can result in relatively benign symptoms such as bruising and petechiae, but ultimately has the potential to cause severe hemorrhage and even death. The causes of thrombocytopenia are multifactorial and fall into one or both of two groups, immune and nonimmune mediated.

Precise identification of the cause of a patient's thrombocytopenia can be challenging to the clinician. A further challenge can be the effective management and treatment of this condition.

The following report will detail a case of platelet refractoriness in a pediatric cardiac surgical patient requiring ECMO.

CASE PRESENTATION

A 19-month-old girl was admitted to the pediatric intensive care unit (PICU) for respiratory support before cardiac surgery. This child's background included an undiagnosed syndrome involving developmental delay, failure to thrive, neonatal hypotonia, arthrogryposis, and small optic nerves. The child also had an extensive cardiac history including coarctation of the aorta, bicuspid aortic valve, atrial septal defect (ASD), and ventricular septal defect (VSD). She underwent a coarctation repair and pulmonary artery (PA) band in the neonatal period, which was complicated postoperatively by a chylothorax and a right internal jugular thrombosis.

Following spontaneous closure of the ASD and VSD, the PA was debanded and augmented at 9 months of age; a subaortic membrane was also resected at this time. Over

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the following 8 months, the child suffered from worsening valvular and subvalvular aortic stenosis, left ventricular mid-cavity obstruction, and mitral stenosis. At 18 months of age, she underwent a Konno procedure, with a 12-mm aortic homograft, a subaortic resection, and mitral valve repair. This time, the postoperative period was complicated by a prolonged PICU stay due to pleural effusions, requiring draining. After eleven postoperative days, she was discharged to the ward.

One month after being discharged to the ward, she was readmitted to the PICU for respiratory support with noninvasive continuous positive airway pressure ventilation and intravenous (IV) diuretic therapy for worsening cardiac failure. The following day, she went to the theater and underwent an aortic root enlargement and aortic valve replacement with a 16-mm mechanical valve, a mitral annular augmentation, and mitral valve replacement with a 16-mm mechanical valve. Cardiopulmonary bypass (CPB) time was 5 hours and 29 minutes, with an aortic cross-clamp time of 3 hours and 56 minutes.

The immediate postoperative period was relatively uneventful. A heparin infusion was commenced on the first postoperative day to maintain an anti-Xa level at .3–.5 u/mL. The child's chest was closed on the third postoperative day. Over the following days, she became increasingly edematous and had periods of low cardiac output state requiring fluid bolus' and adjustment of inotropes.

On the 8th postoperative day, she was successfully extubated. On this same day, it was noted that her platelet count had reduced from $116 \times 10^9/L$ to $56 \times 10^9/L$. The intensivist on duty initially felt that the child may be suffering from heparin-induced thrombocytopenia (HIT). A hematologist was consulted about the possibility of HIT, who felt that a low-grade disseminated intravascular coagulation (DIC) was the probable cause of the reduction in the platelet count. Later that day, the child clinically

deteriorated further, culminating in a seizure and cardiac arrest requiring cardiopulmonary resuscitation, and eventual commencement of ECMO.

Venoarterial ECMO with central cannulation was carried out with a 12-Fr aortic cannula and an 18-Fr right atrial cannula. The circuit was a $\frac{1}{4}'' \times \frac{1}{4}''$ Cortiva™ BioActive Surface (Carmeda AB, Upplands Väsby, Sweden) coating, with a $\frac{3}{8}''$ CentriMag pump (Thoratec, Pleasanton, CA) and a Hilite 2400LT oxygenator (Xenious AG, Heilbronn, Germany). A cardiac index of 2.8–3.0 L/min/m² was maintained throughout the ECMO run. Because of the presence of mechanical valves, the anticoagulation protocol for this child's ECMO run was to maintain an anti-Xa level at .4–.6 u/mL. This was maintained through titration of a heparin infusion directly into the ECMO circuit, throughout the ECMO run.

The child was on ECMO for a total of 7 days. During this time, she continued to be coagulopathic and thrombocytopenic. She required multiple platelet transfusions that were having an increasingly negligible increment on her platelet count (see Figure 1). The hematologist was again consulted about the possibility of HIT. The hematologist still felt that she did not fulfill the HIT criteria and that because of other factors, including a low fibrinogen, a consumptive process was the most likely cause of thrombocytopenia.

On ECMO day three (11 days postoperation), the possibility of antiplatelet antibodies was raised. The following day, a platelet refractoriness test was carried out, whereby the platelet count was measured 30 minutes posttransfusion of pooled platelets and assessed for an incremental rise. No significant rise was seen. The hematologist suggested administering IV immunoglobulin (IVIG) and sending samples for tissue typing to assess for platelet antibodies.

On ECMO day six (14 days postoperation), a unit of human leukocyte antigen (HLA)-matched apheresis

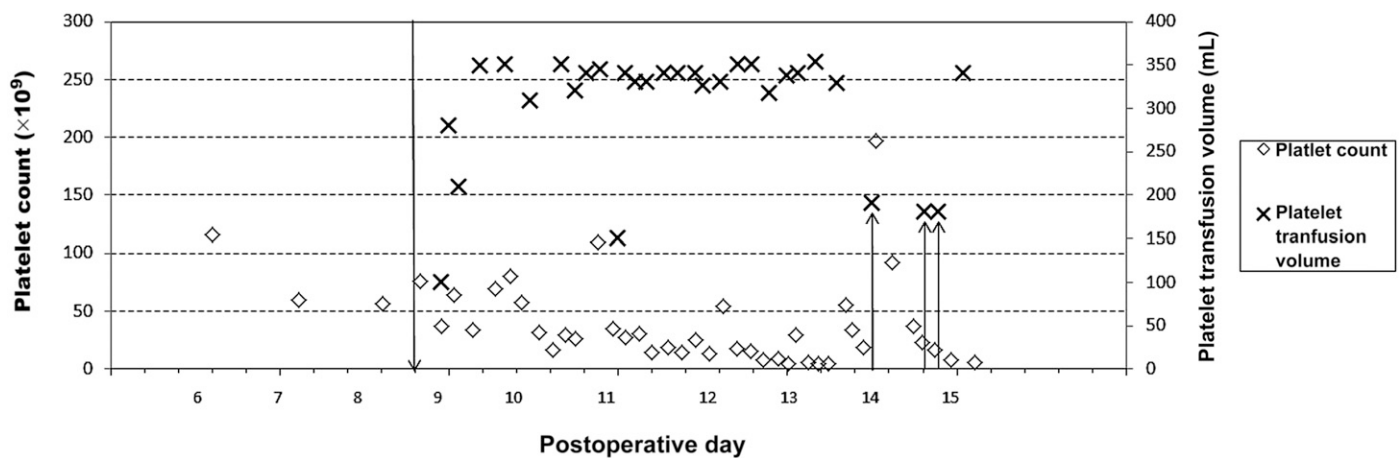


Figure 1. Graph showing the platelet count ($\times 10^9$) in relation to platelet transfusion (mL) with reference to postoperative day. Downward arrow denotes commencement of ECMO. Upward arrows denote transfusion of "HLA-matched" platelets.

platelets was transfused with a significant improvement in the platelet count seen (see Figure 1). The next day, two further units of HLA-matched apheresis platelets (split from a separate single donor) were given without improvement in the platelet count, followed by an unsuccessful wean from ECMO. That night, no further HLA-matched platelets were available, so once again pooled platelets were administered. The following day, after 7 days on ECMO without improvement in cardiac performance, and due to a likely poor prognosis, treatment was withdrawn with family consent and the patient died.

DISCUSSION

This case report details the management of a patient requiring ongoing anticoagulation for mechanical valves and subsequently ECMO, who became thrombocytopenic and ultimately unresponsive to platelet transfusion.

This child underwent four major cardiac procedures. The first procedure was carried out on the second day of life and did not require CPB, but did require heparin exposure. The 2nd, 3rd, and 4th procedures were carried out at 9, 18, and 20 months of age, respectively. The latter three procedures all required CPB, exposure to heparin, and subsequent platelet transfusion. No adverse effects were recorded following heparin exposure at any time. Following platelet administration for the procedures carried out at 9 and 18 months of age, an acceptable incremental rise in platelet count was recorded.

As presented, the possibility of this patient suffering from HIT was brought up twice by the PICU team and was twice thought, by the hematologist, not to be the cause of the patient's thrombocytopenia. Based on the hematologist's opinion, no formal laboratory testing was carried out.

In lieu of formal laboratory testing, if one were to apply the HIT 4Ts scoring system (2) to this case, this patient would be considered an "intermediate" risk of HIT. The patient would have scored highly for the degree of platelet fall and the timing of the platelet fall but lowly for the presence of thrombosis and the potential of other causative factors. Application of this scoring system would have, at the very least, prompted further investigation.

The option of removing all sources of heparin (including changing the ECMO circuit to a nonheparin-coated circuit) and using a heparin alternative was discussed. This was not felt to be an attractive option because of the presence of two mechanical valves in an ECMO patient with a massive blood product requirement and, at that point, increasingly likely diagnosis of immune-mediated platelet refractoriness.

Although this patient was subsequently demonstrated to be suffering from immune-mediated platelet refractoriness, it would appear remiss for HIT to be ruled out without

further testing. Although unlikely, it would be impossible to state with any degree of certainty that this patient did not also have HIT.

Before the diagnosis of immune-mediated thrombocytopenia, and while HIT was being dismissed, the hematologist initially felt DIC as the more likely diagnosis. This was primarily because of a combination of ongoing thrombocytopenia, consistently low fibrinogen, consistently low antithrombin III, and consistently low protein C and S. Because of the patient being on ECMO, the hematologist felt that testing for fibrin degradation products and D-dimers would be nondiscriminatory, so were not measured.

Apart from the measured laboratory values, no evidence of thrombosis existed either within the patient or the ECMO circuit. Although pressure drop is not routinely measured in this ECMO circuit, the relationship between the set revolutions per minute (RPM) and measured flow is observed as a surrogate marker of transmembrane pressure across the oxygenator. During the patient's ECMO run, no deviation between the set RPM and measured flow was observed. Thromboelastograms were also carried out at least twice daily during the patient's time on ECMO. At no point was any evidence of a hypercoagulable state or any secondary fibrinolysis noted.

Generally, several factors may be involved in a patient obtaining a lesser than expected rise in the platelet count following platelet transfusion. The method of storage of the platelets, or the human red cell ABO blood group system (ABO) compatibility, has been described as a factor that may influence the incremental rise in the platelet count posttransfusion (3). Over recent times, inadequate responses to platelet transfusions have decreased with the increased usage of leucodepleted product, but it still occurs and is extremely concerning to the clinician (3).

This patient, as is standard at the authors' institution, received ABO-identical platelets that were leucodepleted and also irradiated. All platelets transfused following this, and previous procedures, were 2–4 days old. The patient, however, remained nearly completely unresponsive to platelet transfusions as demonstrated by poor post-transfusion platelet increments, leading to the diagnosis of platelet refractoriness.

Many formalized (yet not standardized) definitions exist for defining platelet refractoriness. These can range from a lower than expected rise in the platelet count at a set posttransfusion time, right through, to mathematical calculations at set time intervals. In an attempt to bring some order to the myriad of definitions, the Trial to Reduce Alloimmunization to Platelets study (4) defined platelet refractoriness as a 1-hour corrected count increment of less than $5 \times 10^9/L$ on two sequential occasions, using ABO-identical fresh platelets. As detailed earlier, this patient was deemed to be refractory to platelet transfusion because of a poor rise in the platelet count 30 minutes posttransfusion.

Platelet refractoriness can be either immune or nonimmune in nature. Nonimmune-mediated causes may be because of the quality of the platelets, such as inadequate volumes being transfused and platelet age; fresher platelets improve the response to platelet transfusion (5). The patient's clinical condition is also cited as a factor in nonimmune platelet refractoriness. These factors include loss of platelets through bleeding, use of platelets through accelerated consumption (5), sequestration, and decreased production graft-vs.-host disease and certain medications (3).

With regard to the child in this report, nonimmune platelet refractoriness was considered, and although not completely ruled out, it was felt to not be the sole cause of her lack of response to platelet transfusion. The clinical picture of rapid clearance of platelets despite multiple transfusions strongly favored immune-mediated factors being involved.

Immune-mediated causes of platelet refractoriness center on patient alloimmunization due to prior exposure from pregnancy, transfusions, and/or transplantation (3). Alloimmunization to platelet antigens is from either or both the HLA system and the human platelet antigen (HPA) system.

Platelets express HLA Class I A and B antigens. Prior sensitization to these antigens, through transfusion, pregnancy, or transplantation, results in an alloimmune response. Many platelet-specific antigens have been characterized, yet only five of them are known to result in alloimmunization and platelet refractoriness; these five are GPIa, GPIb, GPIIb, GPIIIa, and CD109.3 (6).

Patients who have previously received multiple blood transfusions are said to express HLA antibodies in anywhere from 30 to 70% of cases; it is this immunologically mediated reaction that is understood to be the primary immune cause for platelet refractoriness. The expression of platelet-specific antibodies is considered to be rare in comparison (7).

The patient in this case had had prior exposure to multiple platelet transfusions in both this and prior admissions to hospital. This patient was therefore, at the very least, at increased risk of alloimmune platelet refractoriness.

Clinically, the patient was presenting a serious medical problem. Acutely, these problems were due to actively bleeding yet requiring anticoagulation for ECMO, with potential alloimmune platelet refractoriness. An effective intervention for such patients is the provision of platelets from HLA-matched or HLA-compatible donors (8).

To determine the presence and specificity of antibodies that could affect the survival of transfused platelets, the patient must first be tissue typed. More specifically, this involves determining the HLA type (class I) of the patient and the anti-HLA antibody specificities present in the patient's plasma. Compatible allogeneic platelets may be HLA matched to the patient or lack the antigens to which the patient has demonstrable alloantibodies (8).

Several methods presently exist, in the laboratory, for determining the presence and specificity of antibodies that could affect the survival of transfused platelets. To determine the characteristics of any antibodies present in this patient, the Luminex technology was used. The Luminex technology involves binding soluble antigens to latex or plastic beads; the patient's serum is then incubated with the beads. Any unbound antibody is then washed away, followed by the addition of antihuman globulin with several different fluorochromes, to permit individual classification of each bead. The detection of fluorochrome bound to antihuman globulin would demonstrate the presence of HLA or HPA antibody, with the strength of the antibody being expressed as mean fluorescence intensity (MFI) (6).

When looking for HLA antibodies, the Australian Red Cross Blood Service defines a MFI >8,000 as strong, a MFI >2,000 and <8,000 as moderate, and a MFI <2,000 and >500 as weak. The patient under discussion had three separate HLA antibodies in the strong category, 26 separate HLA antibodies in the moderate category, and 14 separate HLA antibodies in the weak category. No "platelet" antibodies were detected.

These results show that alloimmunization had indeed taken place via the HLA system, but not the HPA system. This would mean that should this patient be exposed to donor platelets containing antigens to which the patient has demonstrable alloantibodies, then an immune-mediated response would take place.

The results from the tissue typing must be obtained before locating a suitably compatible unit of platelets. This process is itself reasonably time consuming. In addition, it can take several more days for the identification and recruitment of a suitable platelet donor (6). One can see how this process could present difficulty in the clinician being able to administer a timely platelet transfusion.

As mentioned, this patient only had an incremental rise in platelet count following one of the three transfusions of "HLA-matched" platelets. When contacted, the Australian Red Cross Blood Service informed us that no platelet donors on the list of platelets awaiting release were HLA compatible; all had an antigen for which the patient had an antibody.

The first platelet donor (after which our patient did have an incremental rise in the platelet count) had one unacceptable antigen with an MFI of 3,293. The second platelet donor, which was a double donation equivalent to two standard doses of platelets (after which our patient did not have an incremental rise in platelet count), had two unacceptable antigens with an MFI of 4,788 and an MFI of 2,614.

The Australian Red Cross Blood Service went on to describe how the platelets issued were the best available in inventory at the point of request, and were dispensed because of the critical need of the child at that time. Had a donor been booked from the child's "call up list," any

collection would not have cleared in time to meet the request.

It would seem that it is both inappropriate and misleading to refer to either of these “HLA-matched” transfusions as “matched.” They may indeed have been the best available at the point of request, but in reality, they were both “mismatched” donors. It is likely that an incremental rise was achieved following the first “matched” transfusion because of the presence of only one unacceptable antigen, whereas the second, split, transfusion of “matched” platelets contained two unacceptable antigens.

If HLA-matched platelets are unavailable, or ineffective, clinical management will become decidedly more challenging. While acknowledging the lack of robust studies in this area, case studies and small uncontrolled studies have reported strategies with apparent merit. Such strategies include cryopreservation of autologous platelets, anti-fibrinolytics, IVIG, plasma exchange, massive platelet transfusion, and HLA-stripped platelets (5).

Although it is beyond the scope of this article to address each of these alternative strategies, the patient under discussion did receive IVIG, on the advice of the hematologist, to ameliorate the poor response to platelets. There are conflicting data on the treatment of platelet transfusion refractoriness with IVIG, with most reviewed articles showing improvement in platelet counts post-platelet transfusion, reduction in bleeding, or decreased platelet transfusion requirements (5). No discernible benefit was seen in the patient in this report following administration of IVIG.

CONCLUSIONS

This case demonstrates the challenges faced by the clinician in dealing with a critically ill child requiring

anticoagulation with immune-mediated, and possibly nonimmune, platelet refractoriness. Once thrombocytopenic, this patient became unable to be effectively managed, despite massive platelet transfusion and administration of IVIG.

The management of nonimmune, immune, and mixed platelet refractoriness is challenging and complex. When platelet refractoriness posttransfusion is diagnosed, the importance of having in place a systematic approach to elucidating its origin cannot be overstated (5). Having an understanding of the causative factors specific to the platelet product, the patient and the possibility of an immune response are essential for the clinician to effectively direct medical therapy.

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