**Title**
Extracorporeal life support survival in a pediatric hematopoietic cellular transplant recipient with presumed GvHD-related fulminant myocarditis.

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Title: **Extracorporeal Membrane Oxygenation Survival in a Pediatric Hematopoietic Cellular Transplant Recipient with Presumed Graft Versus Host Disease-Related Fulminant Myocarditis**

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We report the case of a 15-year-old female with pre-B ALL status post allogeneic hematopoietic stem cell transplantation (HCT) who presented on transplant day +75 with cardiac arrest due to ventricular fibrillation associated with fulminant myocarditis. This case merits discussion due to (1) her survival after 17 days of veno-arterial extracorporeal membrane oxygenation (ECMO) and (2) the possibility of cardiotropic graft versus host disease (GVHD).

The patient was diagnosed with high-risk hypodiploid pre-B ALL notable for 37 chromosomes of the precursor B-cell lineage. She received daunorubicin (75 mg/m$^2$), peg-asparaginase, vincristine, and prednisone for induction therapy, followed by cyclophosphamide, cytarabine, and 6-mercaptopurine for consolidation therapy resulting in MRD-negative first complete remission. Her course was complicated by mild transaminitis and hyperglycemia requiring insulin therapy. Given the high risk of relapse and poor 5-year event-free survival (1), she proceeded to HCT four months after leukemia diagnosis. Standard pre-HCT evaluation was significant for obesity (BMI 37) with biopsy-proven steatohepatitis, but normal heart, lung, and kidney function (EF 61.4%, DLCO 64%, CrCl >150mL/min) and a Karnofsky performance status of 90%. She underwent myeloablative conditioning with busulfan (targeted AUC 80 mg x h/L), fludarabine 10 mg/m$^2$, clofarabine 30 mg/m$^2$, and rabbit anti-thymocyte globulin (10 mg/kg total) and then received a 12/12 HLA-matched unrelated donor peripheral blood HCT. She received acyclovir, caspofungin, TMP-SMX, and IVIG for opportunistic infection prophylaxis, tacrolimus and methotrexate for GVHD prophylaxis, and ursodiol for hepatic venoocclusive disease prophylaxis.

Her early post-transplant course was complicated by *C. difficile* colitis, *E. faecalis* cystitis, *S.epidermidis* bacteremia, and intermittent low-level viremia with adenovirus, CMV, and HHV-6. Her immune reconstitution was marked by neutrophil engraftment on day +16, a pre-discharge ALC of 1600 cells/uL (6% CD4+ T-cells, 66% CD8+ T-cells, 4% CD19+ B-cells, 16% CD56+ NK cells), and 98-100% donor chimerism in all lineages. She was discharged on day +40 in improved state of health with continued tacrolimus for GVHD prophylaxis and cidofovir prophylaxis for recent but resolved adenoviremia. Of note, her tacrolimus level was undetectably low at all subsequent outpatient appointments, suggesting noncompliance.

On transplant day +75, she was found in her bed unresponsive, pulseless, and apneic by her parents. On arrival, EMS identified ventricular fibrillation and defibrillated the patient with temporary return of spontaneous circulation followed by further ventricular fibrillation (**Figure 1**). The patient received further defibrillation, endotracheal intubation, and cardiopulmonary resuscitation during transport to UCSF Benioff Children's Hospital in San Francisco. Initial diagnostic testing showed a troponin-I of 6.68 ug/L (reference range <0.05 ug/L), BNP of 2180 pg/mL (reference <48 pg/mL), cardiomegaly and moderate interstitial edema on chest x-ray, severely depressed biventricular function on echocardiogram (LVEF 27%), and widened QRS with ST and T wave abnormality on electrocardiogram. She continued to have intermittent ventricular fibrillation despite treatment with lidocaine, amiodarone, and electrical defibrillation, and therefore initiated veno-arterial ECMO through her left femoral artery and vein that same day.

In addition to conventional supportive care, she received continuous venovenous hemodialysis due to fluid overload; lidocaine, amiodarone, and metoprolol to treat her arrhythmias~~,~~; vasoactive infusions for heart failure~~,~~; both IVIG and methylprednisolone for presumed myocarditis~~,~~; and a variety of broad-spectrum antimicrobial agents including liposomal cidofovir for possible adenoviral reactivation, given positive adenovirus by PCR in her urine, though her blood remained negative. She underwent exhaustive diagnostic testing in search of infectious, inflammatory, toxin-mediated, and other common and uncommon etiologies of fulminant myocarditis but no etiology was identified. She underwent cardiac catheterization on day +79 during which a balloon atrial septostomy was performed, coronary abnormalities were excluded, and a myocardial biopsy was taken that showed ~~diffuse~~ marked CD8+ lymphocytic infiltration in the setting of severe myocarditis without fibrosis and negative immunohistochemical staining for ~~numerous~~ CMV and adenovirus~~es~~ (**Figure 2**).

The family consented to a research study for the use of unbiased next-generation genomic sequencing for pathogen identification in the myocardial biopsy. Briefly, a 0.5mm formalin-fixed paraffin-embedded block underwent deparaffinization and lysis with xylene, ethanol, proteinase K, and RNase, followed by column-based DNA extraction using the QIAamp FFPE extraction kit (2). DNA then underwent enzymatic repair of formalin-related damage, endonuclease restriction of the 3' tail, ligation of sequencer-specific adaptors and barcodes, and magnetic bead purification with 250-400bp size selection (New England Biolabs), resulting in 18.2 ng of DNA. After sequencing on an Illumina HiSeq 4000 platform, an initial $1.5 \times 10^8$ sequencing pairs underwent quality filtration and duplicate compression, resulting in a final $7.7 \times 10^6$ read pairs. The human genome v38 and contaminants from the water control were then subtracted from these reads using STAR and the remaining 125 base pair sequences were aligned to the NCBI nt and nr databases for a match to any known microbial DNA. No matches to non-human DNA were identified.

Although the inciting cause of the patient's arrhythmias was never identified, her cardiac, pulmonary, and renal function slowly improved. After 17 days of ECMO, she was decannulated on day +92 and then extubated on day +102. Her post-ECMO course was complicated by severe deconditioning resulting in several extubation failures and ultimate reliance on noninvasive BiPAP. She remained on high-dose glucocorticoid for treatment of presumptive GVHD. She improved from a multiorgan perspective but ultimately succumbed to disseminated aspergillosis and passed away on day +156.

There are two noteworthy points meriting discussion. First, we raise the question of a possible cardiotropic graft versus host pathobiology, which has been reported only three times to date (3-5). While our patient's cardiac biopsy identified severe cytotoxic T-cell infiltrates that typically suggest viral myocarditis, all tests for infection were negative. We confirmed this by performing experimental next generation DNA sequencing of the heart biopsy itself, which did not identify any pathogenic microbial DNA. While it is possible that a cardiac infection could have been missed due to biopsy sampling error, there was no evidence of pathogens in other tissue, including nasopharynx, endotracheal aspirate, plasma, and stool, although she did have intermittent adenoviruria. Further, given the fulminant nature of her symptoms and the absence of chronic fibrosis on biopsy, it is unlikely that a subacute infection triggered her myocarditis but resolved prior to detection. Whereas all infectious diagnostics of her myocardium were negative for our patient, several cases of post-HCT fulminant myocarditis attributable to viruses and fungi have been reported (6-9). Importantly, pathogens traditionally detected through serologies would be challenging to diagnose in our patient given the expected B-cell dysfunction post-HCT and the reliance on IVIG for serologic immunity.

Our patient bears strong similarity to two previously described patients (3, 4). Importantly, all three patients received anthracyclines for leukemia therapy, which are known cardiotoxic agents responsible for long-term dose-dependent depression of cardiac function (10). In describing one case of fulminant cardiac GVHD, *Platzbecker et al* posited that anthracycline-induced myocardial injury might lead to upregulated HLA and costimulatory molecule expression in the heart, thus attracting alloreactive T-cells (3). Further, whether intentional for a graft-versus-leukemia effect or unintentional due to medication non-compliance, none of the three patients received GVHD prophylaxis in the months prior to presentation. Post-transplant immune reconstitution in the absence of GVHD prophylaxis is associated with severe cytokinemia and may have been further exacerbated by intermittent viremia in our patient (11).

Interestingly, our patient never developed definitive GVHD of typical organ systems such as the gut, liver, or skin (the latter of which was biopsied twice with negative histology). Although GVHD biomarkers were elevated (ST2 >100 ng/mL, reference range <30; REG3a 256.6 ng/mL, reference range <74.5; elafin 23.7 ng/mL, reference range 5-22.9), these markers have not been validated in the context of multiorgan failure (12). Additional evidence for post-HCT cardiotropic immune dysregulation includes (1) a case report of fulminant myocarditis after autologous HCT combined with IL-2 therapy, and (2) a case report of steroid-responsive complete heart block temporally associated with the development of GVHD in an infant with SCID status post allogeneic HCT (13, 14).

Second, although our patient ultimately died from invasive aspergillosis, she survived a 17-day course of VA-ECMO, which is rare for pediatric HCT patients and invites further conversation on the ~~candidacy~~ utility of ~~HCT patients for~~ extracorporeal life-saving therapies for pediatric HCT patients. Data regarding usage of VA-ECMO for primary cardiac support in this population are rare, particularly when deployed as extracorporeal cardiopulmonary resuscitation. The majority of published data in pediatric HCT patients describe ECMO for respiratory support and attribute the 80-100% mortality to the largely irreversible nature of severe lung injury post-HCT (15-17). Recently, however, some centers have reported survivors and, with both improved patient selection and emerging experimental therapies for post-HCT lung injury, the role of ECMO in pediatric HCT is an evolving field (18-23). Clinicians should be aware of the potential for alloreactive cardiotropic inflammation after allogeneic HCT, particularly in patients with

anthracycline exposure and robust CD8+ reconstitution. Additional research into the pathobiology of this rare but disastrous phenomenon is warranted.

Figure 1) Electrocardiogram Demonstrating Ventricular Fibrillation in a Pediatric Allogeneic Hematopoetic Stem Cell Transplant Patient with Fulminant Myocarditis
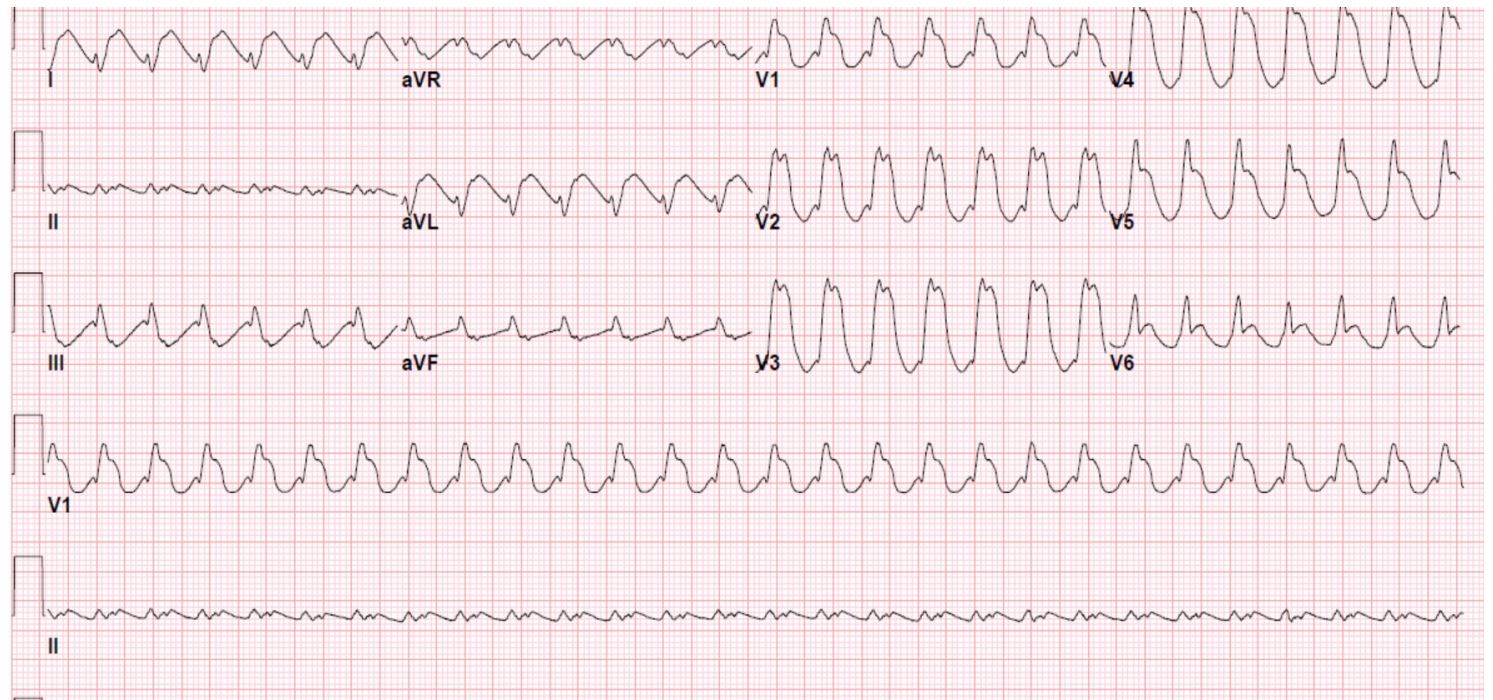


Legend: 12 lead electrocardiogram showing tachycardia, widened QRS, non-specific ST and T segment changes consistent with ventricular fibrillation.

Figure 2)

[hopefully will be pathology slide from the clinical lab]

Legend:

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