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LETTER TO THE EDITOR

# Extracorporeal life support survival in a pediatric hematopoietic cellular transplant recipient with presumed GvHD-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 venoarterial extracorporeal life support (ECLS) and[2] the possibility of cardiotropic 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 minimal residual disease (MRD)-negative first CR. 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 4 months after leukemia diagnosis. Standard pre-HCT evaluation was significant for obesity (body mass index 37) with biopsy-proven steatohepatitis, but normal heart, lung and kidney function (ejection fraction (EF) 61.4%, diffusing capacity (DLCO) 64%, creatinine clearance (CrCl) $>150$ mL/min) and a Karnofsky performance status of 90%. She underwent myeloablative conditioning with busulfan (targeted area under the curve (AUC) 80 mg × h/L), fludarabine 40 mg/m$^2$, clofarabine 120 mg/m$^2$ and rabbit anti-thymocyte globulin 10 mg/kg, and then received a 12/12 HLA-matched unrelated male donor peripheral blood HCT. She received acyclovir, caspofungin, trimethoprim-sulfamethoxazole (TMP-SMX) and IVIg for opportunistic infection prophylaxis; tacrolimus and methotrexate for GvHD prophylaxis; and ursodiol for hepatic veno-occlusive disease prophylaxis.

Her early post-transplant course was complicated by *Clostridium difficile* colitis, *Enterococcus faecalis* cystitis, *Staphylococcus epidermidis* bacteremia, and intermittent low-level viremia with adenovirus, CMV and human herpes virus-6 (HHV-6). Her immune reconstitution was marked by neutrophil engraftment on day +16, a pre-discharge ALC of 1600 cells per μL (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, cidofovir prophylaxis for recently resolved adenoviremia and a recent electrocardiogram showing normal sinus rhythm without abnormality. Between transplant and discharge, she did not undergo further cardiac-specific diagnostics such as b-type natriuretic peptide (BNP) measurement or echocardiography as there was a lack of clinical suspicion for cardiac dysfunction. Of note, her tacrolimus level was undetectably low at all subsequent outpatient appointments, suggesting noncompliance. She was seen in outpatient clinic on transplant day +61 for a routine check, at which point she reported significantly improved activity level, reduction in nausea and general constitutional improvement.

On transplant day +75, she was found in her bed unresponsive, pulseless and apneic by her parents. On arrival, emergency medical services (EMS) identified ventricular fibrillation recalcitrant to electrical defibrillation. The patient underwent endotracheal intubation and cardiopulmonary resuscitation at a referring hospital before transport to UCSF Benioff Children's Hospital in San Francisco. Initial diagnostic testing showed a troponin-I of 6.68 μg/L (reference $<0.05$ μg/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 (left ventricular ejection fraction (LVEF) 27%), and widened QRS with ST and T wave abnormality on electrocardiogram (Figure 1). She continued to have intermittent ventricular fibrillation despite treatment with lidocaine, amiodarone and electrical defibrillation, and therefore initiated venoarterial ECLS through her left femoral artery and vein that same day (CardioHelp, HLS Set 7.0, Maquet, Wayne, NJ, USA).

In addition to conventional supportive care, she received continuous venovenous hemodiafiltration due to fluid overload; lidocaine, amiodarone and metoprolol to treat arrhythmias; vasoactive infusions for heart failure; IVIg and methylprednisolone for presumed myocarditis; and a variety of broad-spectrum antimicrobial agents including liposomal cidofovir for adenoviruria without adenoviremia. 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 coronary abnormalities were excluded, a balloon atrial septostomy was performed due to left atrial hypertension with left ventricular dysfunction, and a myocardial biopsy was taken that showed marked CD8$^+$ lymphocytic infiltration without fibrosis and negative immunohistochemical staining for numerous viruses (Figure 2).

The family consented to a research study of the use of unbiased next-generation genomic sequencing for pathogen identification in the myocardial biopsy. Briefly, a 0.5 mm 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 250–400 bp size selection (New England Biolabs, Ipswich, MA, USA), resulting in 18.2 ng of DNA. After sequencing on an Illumina (San Diego, CA, USA) 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.[3,4] The human genome v38 and contaminants from the no-template ('water') control were then subtracted from these reads, and the remaining 125 bp sequences were aligned to the NCBI nt and nr databases for a match to any known microbial DNA.[5–7] No matches to non-human DNA were identified above 1

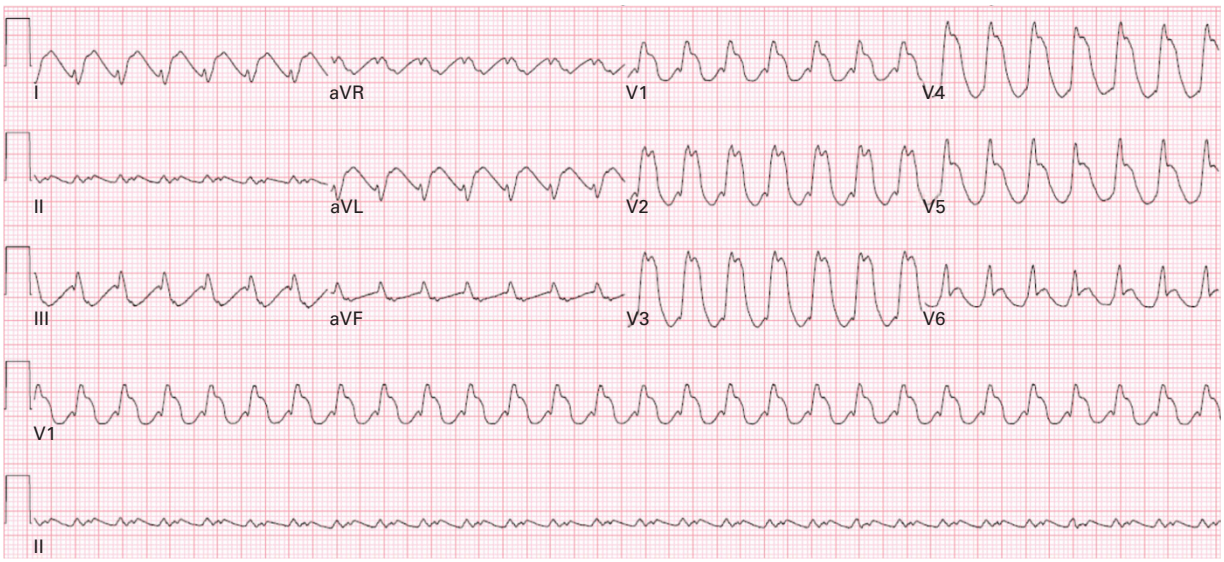


**Figure 1.** Electrocardiogram demonstrating ventricular tachycardia in a pediatric allogeneic hematopoietic stem cell transplant patient with fulminant myocarditis. Twelve lead electrocardiograms showing tachycardia, widened QRS, nonspecific ST and T-segment changes consistent with ventricular tachycardia.

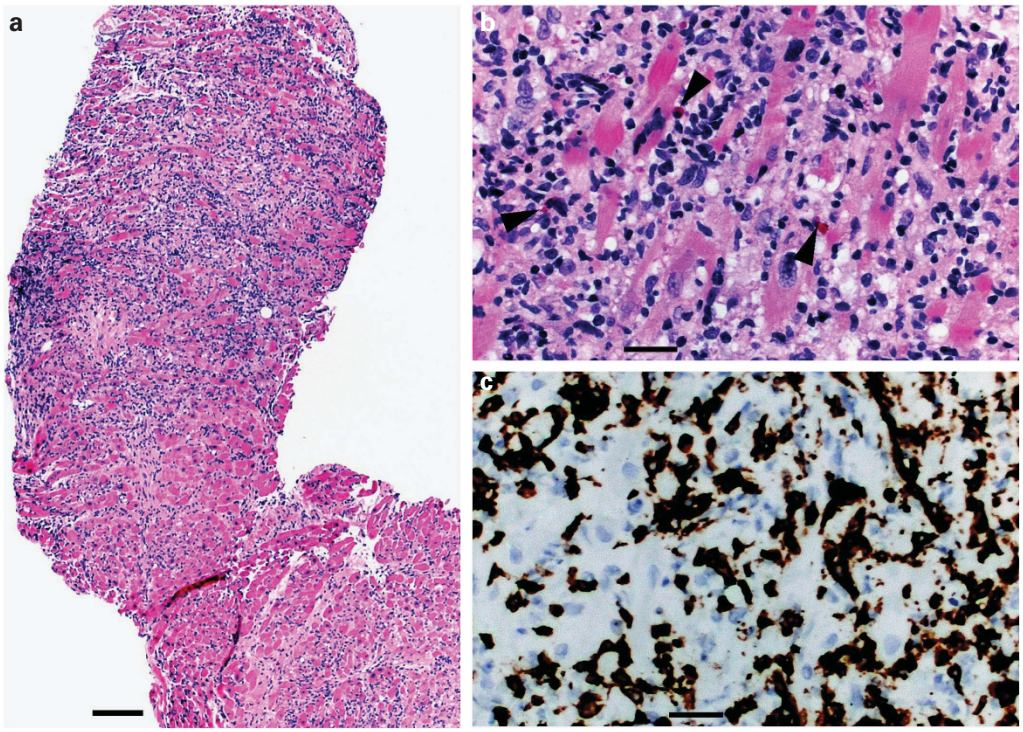


**Figure 2.** Endocardial biopsy demonstrating active myocarditis without fibrosis in a pediatric allogeneic hematopoietic stem cell transplant patient with fulminant myocarditis. (**a**) At low magnification, the myocardium is diffusely inflamed (hematoxylin and eosin, calibration bar = 150 μ). (**b**) High magnification discloses a predominantly mononuclear cell infiltrate with scattered eosinophils (arrowheads; hematoxylin and eosin, calibration bar = 20 μ). Gaps between myofibers signify myocyte damage. (**c**) By immunoperoxidase methods, Ab to CD8 highlights numerous cytotoxic T-lymphocytes with brown reaction product (immunoperoxidase with 3,3′-diaminobenzidine, calibration bar = 20 μ).

read per million sequenced reads. As validation for the presence of donor cells, we identified that 0.40% of the human DNA in the sample uniquely aligned to the Y-chromosome, suggesting a ratio of XY to XX cells of greater than 10:1.[8] This finding is grossly consistent with the lymphocyte:myocyte ratio in the biopsy according to visual inspection at ×40 magnification (Figure 2).

Although the inciting cause of the patient's arrhythmias was never identified, her cardiac, pulmonary and renal function slowly improved. After 17 days of ECLS, she was decannulated on day +92 and then extubated on day +102. Her post-ECLS course was complicated by severe deconditioning resulting in several extubation failures and ultimate reliance on noninvasive Bilevel

positive airway pressure (BiPAP). She remained on high-dose glucocorticoids 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.[9–11] While our patient's cardiac biopsy identified severe cytotoxic T-cell infiltrates that typically suggest viral myocarditis, all tests for viral infection were negative. This finding is consistent with a large case series from Bowles *et al*.[12] in which viral nucleic acid was detected by PCR in only 239 of 624 endomyocardial biopsies from patients with active myocarditis (38%). We confirmed this negative in our patient finding by performing experimental next-generation DNA sequencing of the heart biopsy itself, which did not identify any pathogenic microbial DNA. One caveat is that RNA viruses without DNA intermediates would have escaped this analysis. 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.[13–16]

Our patient bears strong similarity to two previously described patients.[9,10] All three patients received anthracyclines for leukemia therapy, which are known cardiotoxic agents responsible for long-term dose-dependent depression of cardiac function.[17] In describing one case of fulminant cardiac GvHD, Platzbecker *et al*.[9] posited that anthracycline-induced myocardial injury might lead to upregulated HLA and costimulatory molecule expression in the heart, thus attracting alloreactive T-cells. Further, whether intentional for a GvL effect or unintentional due to medication noncompliance, none of the three patients received GvHD prophylaxis in the months before 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.[18]

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 $<30$; REG3a 256.6 ng/mL, reference $<74.5$; elafin 23.7 ng/mL, reference 5–22.9), these markers have not been validated in the context of multiorgan failure.[19] Additional evidence for post-HCT cardiotropic immune dysregulation includes (1) a case report of fulminant myocarditis after autologous HCT combined with interleukin-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.[20,21]

Second, although our patient ultimately died from invasive aspergillosis, she separated from veno-arterial extracorporeal life support (VA-ECLS) after 17 days and did not die from a complication directly attributable to ECLS. In fact, she had largely weaned from noninvasive pulmonary support and was preparing for inpatient rehabilitation therapy before developing fulminant invasive aspergillosis. Reports of VA-ECLS for primary cardiac support in the pediatric HCT population are rare, particularly, when deployed as extracorporeal cardiopulmonary resuscitation. In cases of non-HCT pediatric myocarditis, ~20% of patients will require VA-ECLS, and the decision to deploy this therapy is often hyperacute based on the goal of preventing the progression of cardiac ischemia and multiorgan dysfunction syndrome.[22] However, among cases of non-HCT pediatric fulminant myocarditis requiring VA-ECLS, the natural disease progression is encouraging and indicates that ~61% will recover function and separate from VA-ECLS, whereas 13% will not recover function and require cardiac transplantation, and 26% will die prior to cardiac transplantation.[22] Further, Xiong *et al*.[23] recently performed a meta-analysis of 172 cases of pediatric fulminant myocarditis requiring VA-ECMO and found 62.9% survival to hospital discharge. The use of VA-ECLS for cardiac arrest requires the institutional capacity to rapidly, efficiently and safely deploy this type of support, and centers with this capability have demonstrated a survival advantage in pediatric cardiac arrest relative to conventional cardiopulmonary resuscitation (CPR).[24]

The majority of published data regarding the use of ECLS in pediatric HCT patients describe ECLS for respiratory failure, wherein the 80–100% mortality has been attributed to the largely irreversible nature of severe lung injury post HCT.[25–27] 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 ECLS in pediatric HCT is an evolving field.[28–33] Whereas historical reports of pediatric ECLS documented high rates of hemorrhage and clinically apparent stroke, our patient avoided these complications, an outcome we at least partially attribute to a protocolized approach to anticoagulation and thromboprophylaxis.[34] This report invites further conversation on the candidacy of HCT patients for extracorporeal life-saving therapies. 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. The heart biopsy sequencing file is publicly available at: http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001336.v1.p1.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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