Proteomic analysis of pericardial effusions in hematopoietic stem cell transplant recipients

Hematopoietic stem cell transplant (HSCT) is an established treatment for malignant and non-malignant disorders in children and young adults. Pericardial effusion (PEF) is a potentially life-threatening complication of HSCT that occurs in up to 19% of pediatric patients and is associated with increased mortality due to cardiac tamponade.[1-3] In our clinical practice, most clinically significant PEF occur in conjunction with transplant-associated thrombotic microangiopathy (TA-TMA), though the exact mechanism remains unclear.[4] Other potential risk factors for developing PEF after HSCT include myeloablative conditioning, graft-*versus*-host-disease (GVHD), GVHD prophylaxis, Epstein-Barr virus viremia, and abnormal pre-transplant cardiac function testing.[2-7] Knowledge of PEF mechanisms would guide the use of novel targeted therapies and, combined with diligent screening, may prevent the need for pericardial drain placement in some patients. Our objective was to characterize potential mechanistic pathways in the proteome of pericardial fluid from pediatric HSCT recipients with PEF.

We obtained permission from our institutional review board to retrospectively review the clinical course of seven patients with TA-TMA and PEF with pericardial fluid samples stored in our biorepository. Transplantation demographics, details of TA-TMA diagnosis and management, and details of PEF management and clinical course were collected. Pericardial fluid from these seven HSCT recipients and four control autopsy pericardial fluid samples from available pediatric patients who died from non-cardiac causes were analyzed using an aptamer-based proteomics (SomaScan® 11K Assay) platform. Due to institutional review board restrictions, we were unable to ascertain any other cause of death details in the non-HSCT controls. TA-TMA was prospectively diagnosed using laboratory and clinical diagnostic criteria previously published by Jodele *et al*.[8]

Patient demographics, transplant-related characteristics and the clinical courses of the seven patients with PEF are described in Table 1. All patients were diagnosed with TA-TMA, and the diagnosis was made at a median time of 21 days (range, 2-66 days) from HSCT. Cardiac assessments revealed predominantly sinus rhythm in pre-transplant electrocardiogram (ECG) results, with one instance of sinus tachycardia. Pre-transplant ECG findings included dilated aortic root, mildly thickened mitral valve, and a patent foramen ovale. Pericardial fluid sample collection with drain placement occurred between 4 to 224 days after HSCT following diagnosis by echocardiography. Eculizumab was given to all patients for TA-TMA-directed therapy. Six patients required placement of a pericardial drain and two of those six required placements of a second drain due to fluid re-accumulation. No PEF was classified as malignant on clinical pathology review.

We wanted to characterize potential mechanistic pathways in the proteome of pericardial fluid from HSCT recipients with PEF. Analysis of gene expression and pathway activation revealed significant findings when comparing the post-HSCT PEF cases with normal pericardial fluid collected at autopsy for non-cardiac deaths. A total of 1,271 differentially expressed proteins (DEP) were identified with a *P* value ≤0.05. The volcano plot in Figure 1 illustrates the $\log_2$ fold change against the $-\log_{10}$ *P* value, highlighting several genes with notable expression changes, including *CFHR5, SAA1, LEPROT, APOB, FTMT, TNFRSF25, HSD3B2, M1AP* and *LUM*, suggesting potential involvement in the underlying biological processes leading to PEF. Twenty-seven proteins met more stringent differential expression criteria with an adjusted *P* value (P_{adj}) ≤0.05 (Figure 1). A list of the first 100 DEP is shown in the *Online Supplementary Table S1*.

DEP were then entered in Qiagen Ingenuity Pathway Analysis (IPA) software using a *P* value cutoff of 0.05 and absolute log fold-change cutoff of 0.75 (N=785 proteins). Enriched pathways are shown in Figure 2A using an absolute z-score cutoff of 1.5. The top enriched pathway by *P* value was regulation of insulin-like growth factor transport and uptake by insulin-like growth factor binding proteins (IGFBP; *P*=2.2e-14, z-score=4.3). IGFBP have been previously described as diagnostic markers in malignant pleural and peritoneal effusions,[9] though interestingly none of the effusions in our study were malignant.[10] IGFBP have a recognized role in cell senescence which has been described in endothelial disorders and kidney disease.[11,12] Sensescence occurs following tissue injury and associated inflammation, therefore the IGFBP enrichment observed may support endothelial and/or leukocyte senescence as a contributing factor to PEF biology in HSCT recipients.

Other highly enriched pathways were related to inflammation, including the pathogen-induced cytokine storm signaling pathway, interleukin (IL)-17 signaling, IL-17A signaling in airway cells, IL-17A signaling in fibroblasts, acute-phase response signaling, and complement and coagulation cascades. IPA also generates leading upstream regulators of differentially expressed proteins and identified complement factor 5 (C5; *P*=3.2e-10, z-score=3.3), complement C5a receptor 1 (C5AR1; *P*=1.1e-5, z-score=3.3), IL-6 (*P*=5.9e-16, z-score=3.1) and IL-17RA (*P*=1.6e-7, z-score=2.9) as leading activated upstream regulators. Complement-related upstream regulator protein networks are shown in Figure 2B, C. The observed protein, pathway and upstream regulator changes highlight a novel and complex interplay of IL-17

Table 1. Patient demographics and transplant characteristics.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Sex	Male	Male	Female	Male	Female	Male
Race	White	Black	White	Asian	White	White
Age at HSCT, years	10.1	34.6	0.30	3.76	27.9	0.32
Primary diagnosis	ATRT	AML	Hurler syndrome	Fanconi anemia	AML	Hurler syndrome
Donor type	Autologous	Unrelated	Unrelated	Related	Unrelated	Unrelated
Stem cell source	PBSC	PBSC	Cord	Bone marrow	PBSC	Cord
Degree of match	Auto	8/10	10/10	5/10	10/10	8/10
Conditioning regimen	Carb/TT	Bu/Cy	Bu/Cy	Cy/Flu/TBI	Clof/Mel/TT	Bu/Cy
GVHD prophylaxis	None	TCRαβ^{+}/CD19^{+} Depleted	CSA/MMF	CSA/MMF/PTCY	ABA/CSA/MTX	CSA/MMF
Day 100 acute GVHD score	NA	4	0	0	2	1
Chronic GVHD	NA	No	No	No	Yes	No
TA-TMA onset, day from HSCT	66	2	21	20	50	40
TA-TMA risk category	High	High	Moderate	High	High	High
Pre-transplant ECG results	Sinus rhythm	Sinus rhythm	Sinus rhythm	Sinus tachycardia	Sinus rhythm	Sinus rhythm
Pre-transplant echocardiogram results	Normal	Dilated aortic root	Mildly thickened mitral valve with mild prolapse; mild to moderate regurgitation	Normal	Normal	Mildly dilated aortic root, patent foramen ovale (left to right shunting)
Day of initial PEF diagnosis from HSCT	67	-23	3	16	207	84
Pericardiocentesis, day from HSCT	67	4	81	29	224	88
Post-transplant initial PEF size description on echo	Large	Trivial to small	Trivial	Moderate	None visualized by echo, seen during autopsy	Large
Presence of tamponade physiology on echo prior to pericardiocentesis	Yes	Yes	No	Yes	No	Yes
Medical interventions used for PEF and TA-TMA	Eculizumab	Eculizumab	Eculizumab	Eculizumab	Eculizumab	Eculizumab, methylprednisolone
Complications related to PEF	Required 2 drains due to re-accumulation of fluid after first drain removed	None	Required 2 drains due to re-accumulation of fluid after first drain removed	None	None	None
Total length of time pericardial drain(s) in place, days	8	2	32	12	NA	4
Cause of death	Alive	Multi-organ failure due to bacterial infection, TA-TMA and GVHD	Alive	Multi-organ failure due to adenovirus infection	Cardiorespiratory failure	Alive

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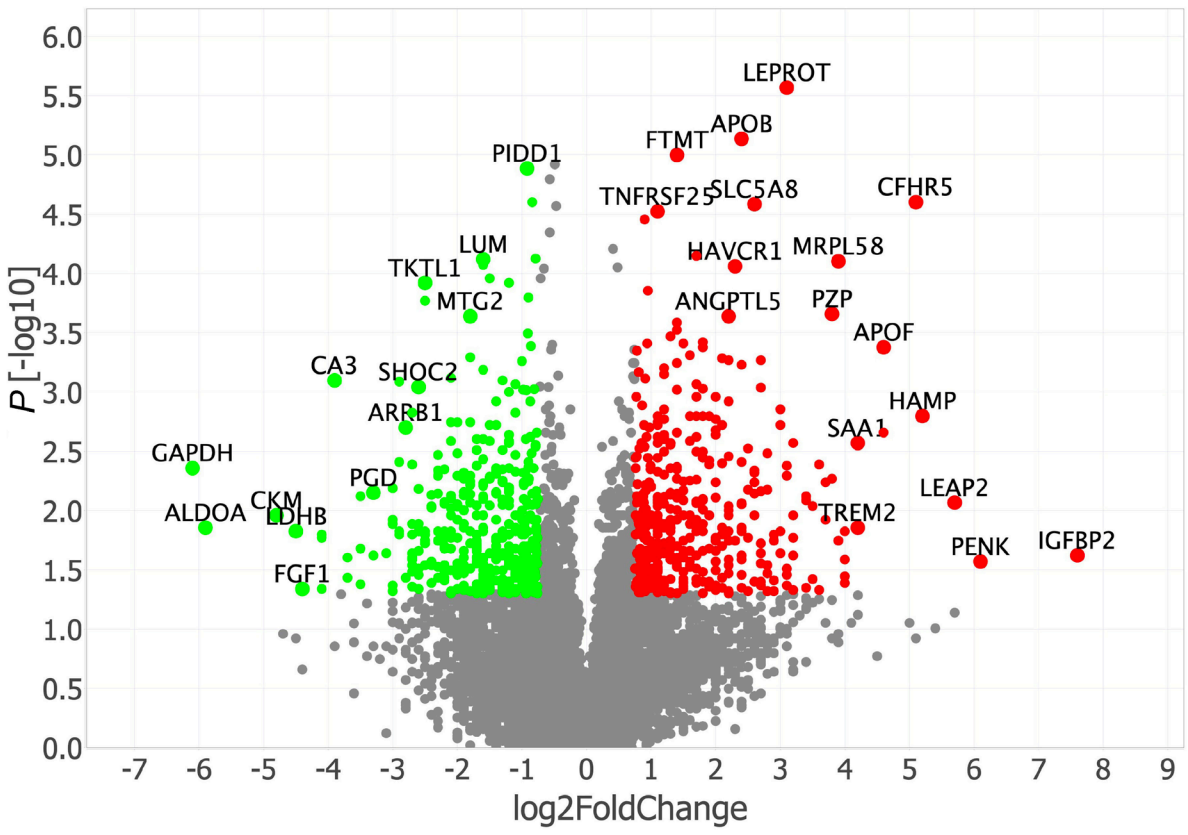
ATRT: atypical teratoid rhabdoid tumor; AML: acute myeloid leukemia; PBSC: peripheral blood stem cell; Carb/TT: carboplatin/thiotepa; Bu/Cy: busulfan/cyclophosphamide; Cy/Flu/TBI: cyclophosphamide/fludarabine/total body irradiation; Clof/Mel/TT: clofarabine/melphalan/thiotepa; GVHD: graft-*versus*-host disease; NA: not applicable; CSA/MMF: cyclosporine/mycophenolate mofetil; ABA/CSA/MTX: abatacept/cyclosporine/methotrexate; TA-TMA: transplant-associated thrombotic microangiopathy; HSCT: hematopoietic stem cell transplant; PEF: pericardial effusion; ECG: electrocardiogram; PTCY: posttransplant cyclophosphamide.

and complement-related pathways in HSCT recipient PEF. Complement factors are known to affect the IL-17 axis, which makes it plausible that these complement and IL-17 findings are both true and potentially related.[13]

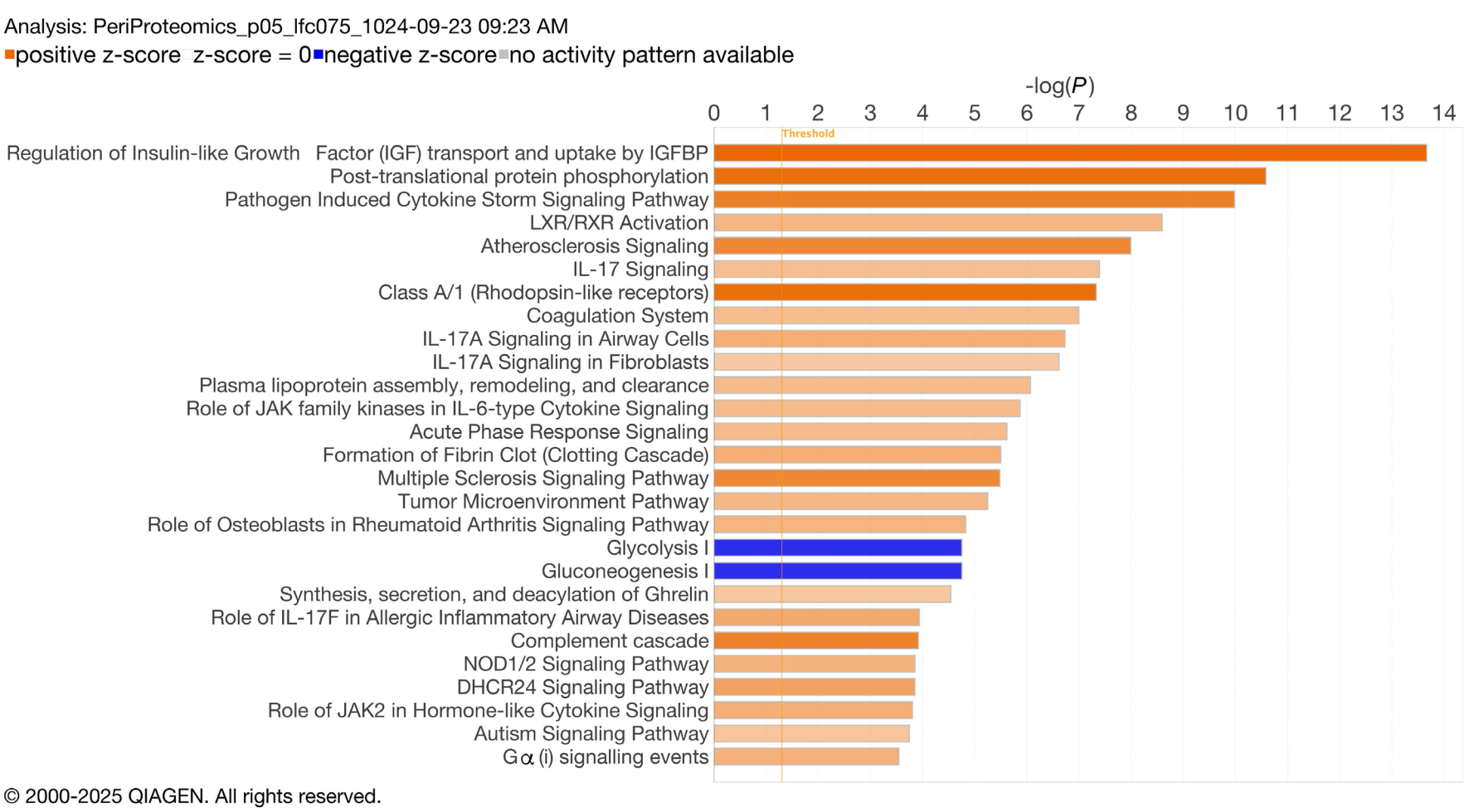
Clinically, we have previously reported that PEF after HSCT frequently occurs in the setting of TA-TMA.[14] Based on this, we focused our analysis on pathways relevant to TA-TMA, specifically complement and coagulation cascades, and identified IL-17 as a potential upstream mediator. This is biologically plausible, as IL-17 is known to promote endothelial activation, enhance vascular permeability, and prime the endothelium for pro-thrombotic states-hallmarks of TA-TMA. IL-17 also promotes endothelial cell senescence, which creates a mechanistic link to IGFBP dicussed above.[15] Additionally, IL-17 has been shown to upregulate C3 expression and drive terminal complement activation in both endothelial and epithelial cells, supporting its role in complement-mediated endothelial injury.[13] Consistent with this, we found that CFHR5, was one of the strongest differentially expressed proteins in our experiment. This finding aligns with the broader enrichment of complement and coagulation pathways observed in Figure 2, further implicating IL-17 driven complement dysregulation in the pathogenesis of PEF after HSCT. CFHR5 functions to protect the body from complement-mediated injury and mutations in this gene have been linked to CFHR5 nephropathy as well as TA-TMA.[16,17] The observed increase in CFHR5 may represent a host protective response to overactive complement activation in TA-TMA. Alternatively, this could indicate an underlying functional polymorphism in *CFHR5* that may predispose these patients to TA-TMA and even PEF. Our prior study of complement gene polymorphisms in TA-TMA identified four TA-TMA patients with *CFHR5* polymorphisms which was the second most commonly mutated gene observed.[18] The association between IL-17, IGFBP, complement proteins and PEF therefore merits further study.

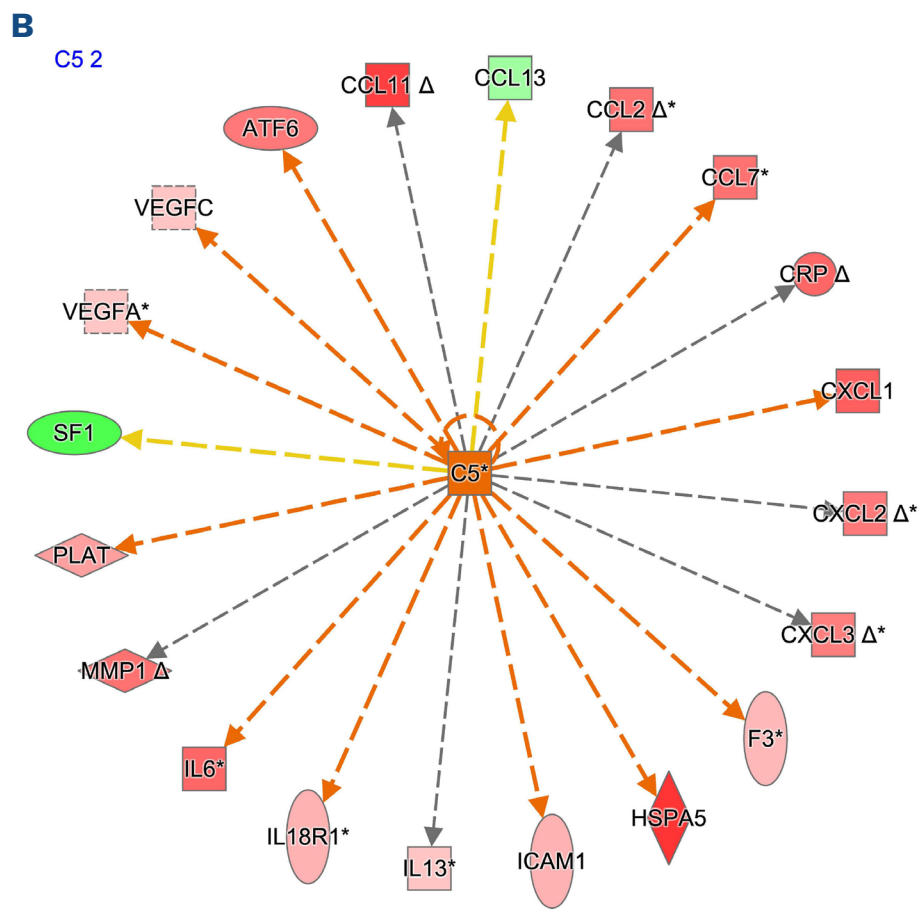
The data presented in this report are important for clinicians managing pediatric HSCT recipients, as PEF is a known complication with significant morbidity. All HSCT recipients in this study were diagnosed with TA-TMA and treated with a C5 inhibitor, therefore the effect of this therapy on PEF after HSCT is not testable in our study cohort. However, complement pathway enrichment was still strong in the pericardial fluid proteome despite C5



Symbol	Log Ratio	*P*	q value
LEPROT	3.1	2.7E-06	0.029
APOB	2.4	7.3E-06	0.03
FTMT	1.4	0.00001	0.03
SHC2	-0.49	0.000012	0.03
PIDD1	-0.92	0.000013	0.03
CD2BP2	-0.57	0.000016	0.03
CFHR5	5.1	0.000025	0.03
NEUROD1	-0.84	0.000025	0.03
SLC5A8	2.6	0.000026	0.03
ST6GALNAC4	-0.47	0.000027	0.03
TNFRSF25	1.1	0.00003	0.03
APBB3	0.9	0.000035	0.032
HEXIM1	-0.57	0.000045	0.038
ARHGAP29	0.41	0.000062	0.046
HSD3B2	1.7	0.000071	0.046
BRD1	-0.79	0.000075	0.046
LUM	-1.6	0.000076	0.046
MRPL58	3.9	0.000079	0.046
M1AP	-1.6	0.000085	0.046
HAVCR1	2.3	0.000087	0.046
IL10RA	0.48	0.000089	0.046
SYT17	-0.66	0.000091	0.046
RIPK3	-1.5	0.00011	0.05
RNF157	-0.71	0.00011	0.05
RABIF	-1.2	0.00012	0.05
PPBP	-2.4	0.00012	0.05
TKTL1	-2.5	0.00012	0.05

Figure 1. Volcano plot of differentially expressed proteins in pericardial fluid from hematopoietic stem cell transplant recipients with pericardial effusions. This volcano plot displays the $\log_2$ fold change (x-axis) *versus* $-\log_{10}$ *P* value (y-axis) for all proteins analyzed. A total of 1,271 proteins were differentially expressed with $P \leq 0.05$. Red and green dots indicate significantly upregulated and downregulated proteins, respectively. Several proteins demonstrated marked differential expression highlighting potential mechanistic pathways. A subset of 27 differentially expressed proteins met stricter criteria with adjusted *P* values ≤0.05.





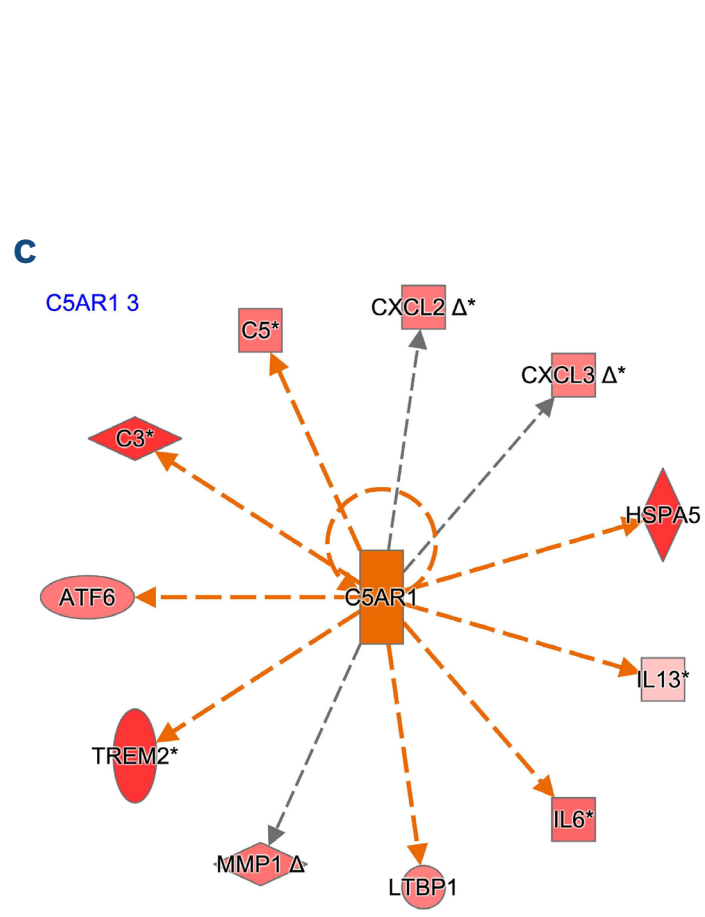


Figure 2. Pathway enrichment and upstream regulator analysis of differentially expressed proteins in pericardial fluid from hematopoietic stem cell transplant recipients with pericardial effusions. (A) Top enriched canonical pathways identified by Ingenuity Pathway Analysis from 785 proteins with $P \leq 0.05$ and absolute $\log_2$ fold change ≥0.75. Pathways are ranked by -log(*P* value), and activity is predicted by z-score (orange: activated, blue: inhibited). The most significantly enriched pathway was "Regulation of insulin-like growth factor transport and uptake by insulin-like growth factor binding proteins," followed by several inflammation-related pathways including interleukin (IL)-17 signaling, cytokine storm signaling, acute phase response, and complement cascade. (B, C) Predicted upstream regulator networks derived from Qiagen Ingenuity Pathway Analysis. Complement factors and cytokines were identified as leading upstream regulators, including C5, C5AR1, IL6, and IL-17RA. Network visualizations show predicted regulatory relationships and directionality of protein expression changes. These findings suggest a central role for complement and IL-17 pathways in the pathogenesis of pericardial effusions following hematopoietic stem cell transplant.

inhibition. Our novel observations involving IL-17 and IGFBP pathways therefore merit further mechanistic study as potential targets of pharmacologic intervention outside of the complement system. IL-17 and IGFBP pathways may represent a unique mechanism of inflammation in the pericardial space of HSCT recipients that is linked to complement system activation. We acknowledge there are limitations to this study including the small sample size and use of normal pericardial fluid and not PEF from non-HSCT subjects as controls. While this limits the ability to differentiate the mechanism of HSCT PEF from non-HSCT PEF, we were still able to identify differentially expression proteins and enriched pathways in HSCT PEF. Pericardial fluid specimens from HSCT recipients without effusions were understandably not available.

In conclusion, rapidly growing PEF can quickly lead to life-threatening complications and the need for invasive procedures. Our study is the first to shed light on the mechanisms of PEF in HSCT recipients and identified targetable pathways and proteins for future study and validation.

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https://doi.org/10.3324/haematol.2025.289076

Received: September 3, 2025.
Accepted: October 31, 2025.
Early view: November 6, 2025.

Disclosures

No conflicts of interest to disclose.

Contributions

JK, AS and SMD conceptualized and supervised the overall study. JK, AS and SMD wrote and revised the manuscript. AW performed statistical analysis and revised the manuscript. KEL and NL assisted with data acquision and revised the manuscript. CD, SJ and TDR revised and revised the manuscript.

Data-sharing statement

Requests for data can be made directly to the corresponding author.

References

1. Chen X, Zou Q, Yin J, et al. Pericardial effusion post transplantation predicts inferior overall survival following allo-hematopoietic stem cell transplant. Bone Marrow Transplant. 2016;51(2):303-306.
2. Tong K, Meng Y, Zhang L, et al. Retrospective study of risk factors for pericardial effusion after haematopoietic stem cell transplantation in children. Hematology. 2023;28(1):2245259.
3. Aldoss O, Gruenstein DH, Bass JL, et al. Pericardial effusion after pediatric hematopoietic cell transplant. Pediatr Transplant. 2013;17(3):294-299.
4. Lerner D, Dandoy C, Hirsch R, Laskin B, Davies SM, Jodele S. Pericardial effusion in pediatric SCT recipients with thrombotic microangiopathy. Bone Marrow Transplant. 2014;49(6):862-863.
5. Westbroek ML, Rahim MQ, Ross MM, Rahrig AL. A fluid relationship: calcineurin inhibitors and pericardial effusions. Pediatr Transplant. 2024;28(1):e14672.
6. Tinianow A, Gay JC, Bearl DW, Connelly JA, Godown J, Kitko CL. Pericardial effusion following hematopoietic stem cell transplantation in children: incidence, risk factors, and outcomes. Pediatr Transplant. 2020;24(5):e13748.
7. Dandoy CE, Davies SM, Hirsch R, et al. Abnormal echocardiography 7 days after stem cell transplantation may be an early indicator of thrombotic microangiopathy. Biol Blood Marrow Transplant. 2015;21(1):113-118.
8. Jodele S, Davies SM, Lane A, et al. Diagnostic and risk criteria for HSCT-associated thrombotic microangiopathy: a study in children and young adults. Blood. 2014;124(4):645-653.
9. Amon LM, Law W, Fitzgibbon MP, et al. Integrative proteomic analysis of serum and peritoneal fluids helps identify proteins that are up-regulated in serum of women with ovarian cancer. PLoS One. 2010;5(6):e11137.
10. Olchovsky D, Shimon I, Goldberg I, et al. Elevated insulin-like growth factor-1 and insulin-like growth factor binding protein-2 in malignant pleural effusion. Acta Oncol. 2002;41(2):182-187.
11. Wang WG, Sun WX, Gao BS, Lian X, Zhou HL. Cell cycle arrest as a therapeutic target of acute kidney injury. Curr Protein Pept Sci. 2017;18(12):1224-1231.
12. Wang Y, Lim R, Nie G. Elevated circulating HtrA4 in preeclampsia may alter endothelial expression of senescence genes. Placenta. 2020;90:71-81.
13. Lajoie S, Lewkowich IP, Suzuki Y, et al. Complement-mediated regulation of the IL-17A axis is a central genetic determinant of the severity of experimental allergic asthma. Nat Immunol. 2010;11(10):928-935.
14. Dandoy CE, Tsong WH, Sarikonda K, McGarvey N, Perales MA. Systematic review of signs and symptoms associated with hematopoietic stem cell transplantation-associated thrombotic microangiopathy. Transplant Cell Ther. 2023;29(4):282.e1-282.e9.
15. Zhang L, Liu M, Liu W, et al. Th17/IL-17 induces endothelial cell senescence via activation of NF-kappaB/p53/Rb signaling

pathway. Lab Invest. 2021;101(11):1418-1426.
16. Bu F, Borsa NG, Jones MB, et al. High-throughput genetic testing for thrombotic microangiopathies and C3 glomerulopathies. J Am Soc Nephrol. 2016;27(4):1245-1253.
17. Menotti S, Donini M, Pessolano G, et al. Atypical hemolytic uremic syndrome: unique clinical presentation linked to rare CFHR5 mutation. EJHaem. 2021;2(4):838-841.
18. Jodele S, Zhang K, Zou F, et al. The genetic fingerprint of susceptibility for transplant-associated thrombotic microangiopathy. Blood. 2016;127(8):989-996.