Temporal transcriptomic profiling of pediatric septic shock patients based upon PERSEVERE-II risk strata identifies a conserved network of cell cycle genes in high-risk patients

Leland Dunwoodie, MD[1]; Mihir Atreya, MD, MPH[2]; Andrew J. Lautz, MD[2]
[1]Department of Pediatrics and [2]Division of Critical Care Medicine, Cincinnati Children's Hospital Medicine Center

Background

Sepsis is a leading cause of death in children worldwide. Yet, personalized therapies beyond antimicrobials and intensive organ support remain elusive in large part due to the heterogeneity amongst children with sepsis. To help unravel this heterogeneity, the **Pediatric Sepsis Biomarker Risk Model (PERSEVERE)-II** was developed and prospectively-validated to identify children with sepsis at high (16-57%) and low (< 2%) risk for 28-day mortality based upon protein biomarker expression and platelet count at PICU admission. However, **it is not known why high-risk children have poorer outcomes over time compared to low-risk children.** Given the complexity of the host immune response, these poorer outcomes are likely driven by intricate gene networks as opposed to a few individual genes.

Objective

To identify genes co-expressed in high-risk patients on Day 1 and Day 3 of PICU admission to reveal conserved gene networks associated with the high-risk stratum, thereby suggesting genetic drivers of mortality in high-risk patients.

Methods

1. The whole blood mRNA of children with septic shock on Day 1 (81 patients; 58 low-risk and 23 high-risk) and Day 3 (71 patients; 53 low-risk and 18 high-risk) of PICU admission was obtained.
2. Raw mRNA counts were normalized with TMM normalization and genes with low counts were removed.
3. A gene co-expression network was created using WGCNA for both day 1 and day 3 expression data.
4. DAVID was used for functional annotation analysis.

Results

Four gene co-expression modules were identified in both the day 1 and day 3 gene co-expression networks that were significantly associated with either the high- or low-risk stratum ($p < 0.05$). **A 198-gene Day 1 module and a 260-gene Day 3 module shared 190 genes; both of these modules were over-expressed in and associated with the high-risk stratum. In addition, both of these modules are cell cycle-related modules,** with "Cell Cycle" being their most enriched functional annotation and > 150 genes being in the "Cell Cycle" Reactome pathway.

Figure 1. Weighted Gene Correlation Network Analysis (WGCNA) was used to identify gene co-expression modules with co-expression relationships enriched for either the high-risk (red) or low-risk (blue) stratum using TMM-normalized Day 1 (top) and Day 3 (bottom) gene expression data. **The Day 1 Yellow Module and Day 3 Yellow Module share 190 genes and are highlighted.** R^2 value correlating each module with PERSEVERE-II risk strata is shown and asterisk denotes p-value showing the significance of said correlation (*** < 0.001, ** < 0.01, * < 0.05).

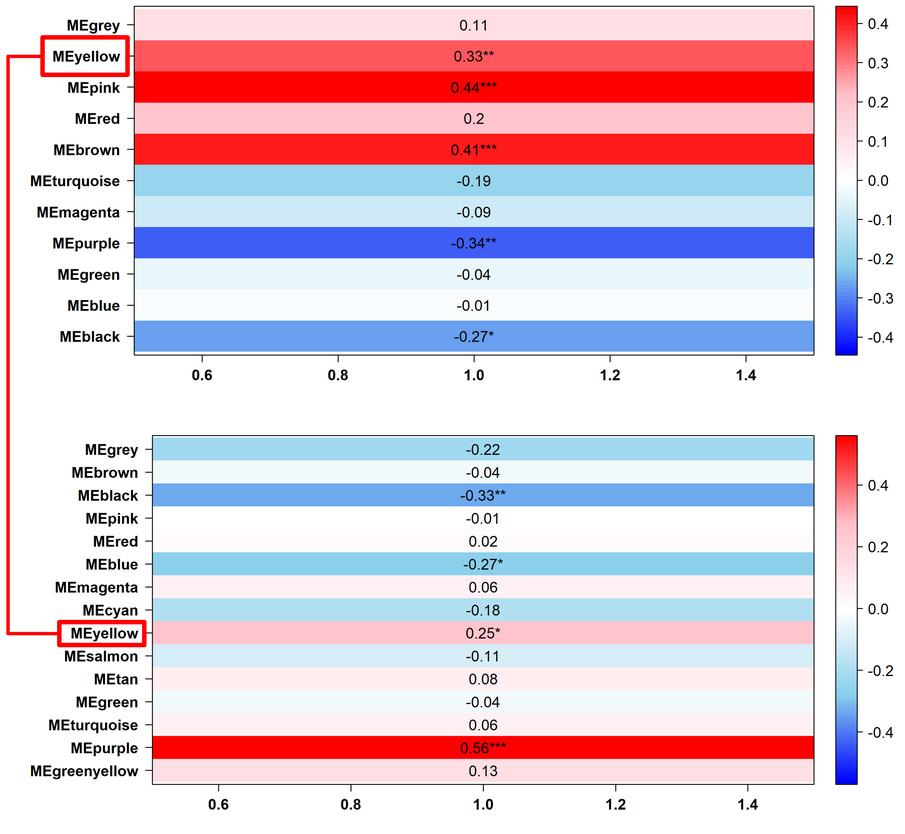

Table 1. The 190 genes shared between the Day 1 Yellow Module and the Day 3 Yellow Module. These genes are over-expressed in high-risk patients and their co-expression relationships are enriched for the high-risk stratum.

ANLN	CIT	HIST1H2AJ	KIF11	RACGAP1
ARHGAP11A	CKAP2L	HIST1H2AL	KIF14	RAD51
ASPM	CKS1B	HIST1H2BB	KIF15	RAD51AP1
ATAD2	CKS2	HIST1H2BC	KIF18A	RRM2
ATAD5	CLSPN	HIST1H2BD	KIF18B	SGO2
AURKA	DEPDC1	HIST1H2BE	KIF20A	SHCBP1
AURKB	DEPDC1B	HIST1H2BF	KIF23	SKA1
BIRC5	DIAPH3	HIST1H2BG	KIF2C	SKA2
BRCA2	DLGAP5	HIST1H2BH	KIF4A	SLC27A2
BRIP1	DNA2	HIST1H2BI	KIFC1	SMC2
BUB1	DSCC1	HIST1H2BJ	KNL1	SPAG5
BUB1B	DTL	HIST1H2BK	KPNA2	SPC24
CCNA2	DUT	HIST1H2BL	MAD2L1	SPC25
CCNB1	E2F7	HIST1H2BM	MCM10	STMN1
CCNB2	E2F8	HIST1H2BN	MCM2	TCF19
CCNE1	ESCO2	HIST1H2BO	MCM4	TICRR
CCNF	ESPL1	HIST1H3A	MCM6	TIMELESS
CDC20	EXO1	HIST1H3B	MELK	TK1
CDC25A	EZH2	HIST1H3C	MKI67	TOP2A
CDC45	FAM83D	HIST1H3F	MND1	TPX2
CDC6	FANCI	HIST1H3G	MYBL2	TRIP13
CDCA2	FBXO5	HIST1H3H	NCAPG	TROAP
CDCA3	FEN1	HIST1H3I	NCAPG2	TTK
CDCA5	FOXM1	HIST1H3J	NCAPH	TYMS
CDCA8	GINS1	HIST1H4A	NDC80	UBE2C
CDK1	GINS2	HIST1H4B	NEK2	UBE2S
CDKN3	GTSE1	HIST1H4C	NOSTRIN	UBE2T
CDT1	H2AFX	HIST1H4D	NUF2	WDHD1
CENPA	HASPIN	HIST1H4F	ORC1	WDR62
CENPE	HELLS	HIST1H4H	ORC6	WDR76
CENPF	HIST1H1B	HIST1H4I	PARPBP	XRCC2
CENPI	HIST1H1C	HIST1H4L	PBK	ZNF367
CENPM	HIST1H1D	HIST2H2AB	PCLAF	ZWILCH
CENPU	HIST1H1E	HIST2H2BF	PCNA	ZWINT
CENPW	HIST1H2AB	HIST2H3D	PLK1	
CEP55	HIST1H2AE	HJURP	PLK4	
CHAF1A	HIST1H2AG	HMGB3	POLQ	
CHEK1	HIST1H2AH	HMGN2	PRR11	
CIP2A	HIST1H2AI	HMMR	PTTG1	

Table 2. **The Reactome pathways most enriched for the 190 shared genes provide evidence for cell cycle-related functions.**

1. Cell Cycle	**3. Cell Cycle Checkpoints**
2. Cell Cycle, Mitotic	**4. M Phase**

Conclusions

190 genes, many of which function in cell proliferation, were co-expressed and over-expressed on both Day 1 and Day 3 of PICU admission for septic shock in high-risk patients. These genes warrant further investigation as potential drivers of the high-risk phenotype.