SUPPLEMENTAL MATERIALS

IFN-γ Signature in the Plasma Proteome Distinguishes Pediatric Hemophagocytic Lymphohistiocytosis from Sepsis and SIRS

Running Title: IFN-γ Distinguishes HLH from Sepsis and SIRS

Lin et al.

Table 1. Clinical Summary of Study Cohorts

A. Patient Cohorts

	Training	Validation	p-value
HLH	21	19	
Sepsis	13	13	
SIRS	10	11	
Total	44	43	0.9

B. Training and Validation Cohorts

		Training	Validation	p-value
Gender	Male	10	8	1.0
	Female	11	11	
Status	Alive	13	12	1.0
	Dead	8	7	
Age (years)	0-3	11	7	0.3
	3-18	9	12	
	18+	1	0	
HLH Category	Primary	18	16	1.0
	Secondary	3	3	
Total		21	19	

Supplemental Table 1. Diagnostic Criteria for HLH, SIRS, Sepsis and Severe Sepsis

A. HLH-2004 Diagnostic Criteria (Adapted from Henter *et al., PBC* 2007)

Clinical Criteria for Diagnosis of HLH
HLH diagnosis is established with at least <u>five</u> of the following:
Fever
Splenomegaly
Cytopenias in at least two cell lines:
Hemoglobin <90 g/L
Platelets <100 × 10^9/L
Neutrophils <1 × 10^9/L
Hypertriglyceridemia and/or hypofibrinogenemia:
Fasting triglycerides >3 mmol/L (>265 mg/dL)
Fibrinogen <1.5 g/L
Hemophagocytosis in marrow or spleen or lymph nodes
Low or absent activity of natural killer cells
Ferritin >500 mcg/L
Soluble cD25 (soluble interleukin-2 receptor) >2400 units/mL (or 2 SD above normal for age)

B. Definitions of Systemic Inflammatory Response Syndrome (SIRS), Sepsis, and Severe Sepsis

(Adapted from Goldstein *et al., PCCM* 2005)

Definition of Systemic Inflammatory Response Syndrome

SIRS is established with at least two of the following, one must be abnormal temperature or leukocyte count

Core temperature of >38.5 or <36^{o}C

Tachycardia (>2 SD above normal for age) in the absence of external stimuli ,chronic drugs, or painful stimuli;

-OR otherwise unexplained persistent elevation over a 0.5-4 hour time period

-OR for children <1 year, bradycardia (mean heart rate <10th percentile for age) in absence of external vagal stimulus, beta-blocker drugs or congenital heart disease

-OR otherwise unexplained persistent depression over a 0.5 hr time period

Tachypnea (>2SD above normal for age)

-OR mechanical ventilation for an acute process not related to underlying neuromuscular disease or the receipt of general anesthesia.

Leukocyte count elevated or depressed for age (not secondary to chemotherapy)

-OR >10% immature neutrophils

Definition of Sepsis

SIRS in the presence or as a result of suspected or proven infection

Definition of Severe Sepsis

Sepsis plus one of the following:

Cardiovascular organ dysfunction

-OR respiratory failure

-OR ≥ 2 other organ system dysfunction (See **Supplemental Table 1C** below)

C. Organ Dysfunction Criteria (Adapted from Goldstein *et al PCCM* 2005)

Cardiovascular

Despite ≥40 mL/kg of intravenous fluid bolus in 1 hr:

- Hypotension: defined as <5th percentile for age or systolic BP <2 SD below normal for age OR
- Need for inotropes/vasopressors to maintain BP in normal range OR
- 2 of the following:
  - Unexplained metabolic acidosis: base deficit >5.0 mEq/L
  - Increase arterial lactate >2x upper limit of normal
  - Oliguria: urine output <0.5 mL/kg/hr
  - Prolonged capillary refill: >5 seconds

Respiratory

- PaO2/FIO2 <300 in absence of cyanotic heart disease or preexisting lung disease OR
- PaCO2 >65 torr or 20 mmHg over baseline PaCO2
- Proven need or >50% FiO2 to maintain saturation ≥92% OR
- Need for nonelective invasive or noninvasive mechanical ventilation

Neurologic

- Glasgow Coma Score (GCS) ≤11 OR
- Acute change in mental status with a GCS ≥3 points from abnormal baseline

Hematologic

- Platelet count <80,000/mm3 or a decline of 50% from highest value recorded over the past 3 days OR
- International normalized ratio >2

Renal

- Serum creatinine >2 times upper limit of normal for age OR
- 2-fold increase in baseline serum creatinine

Hepatic

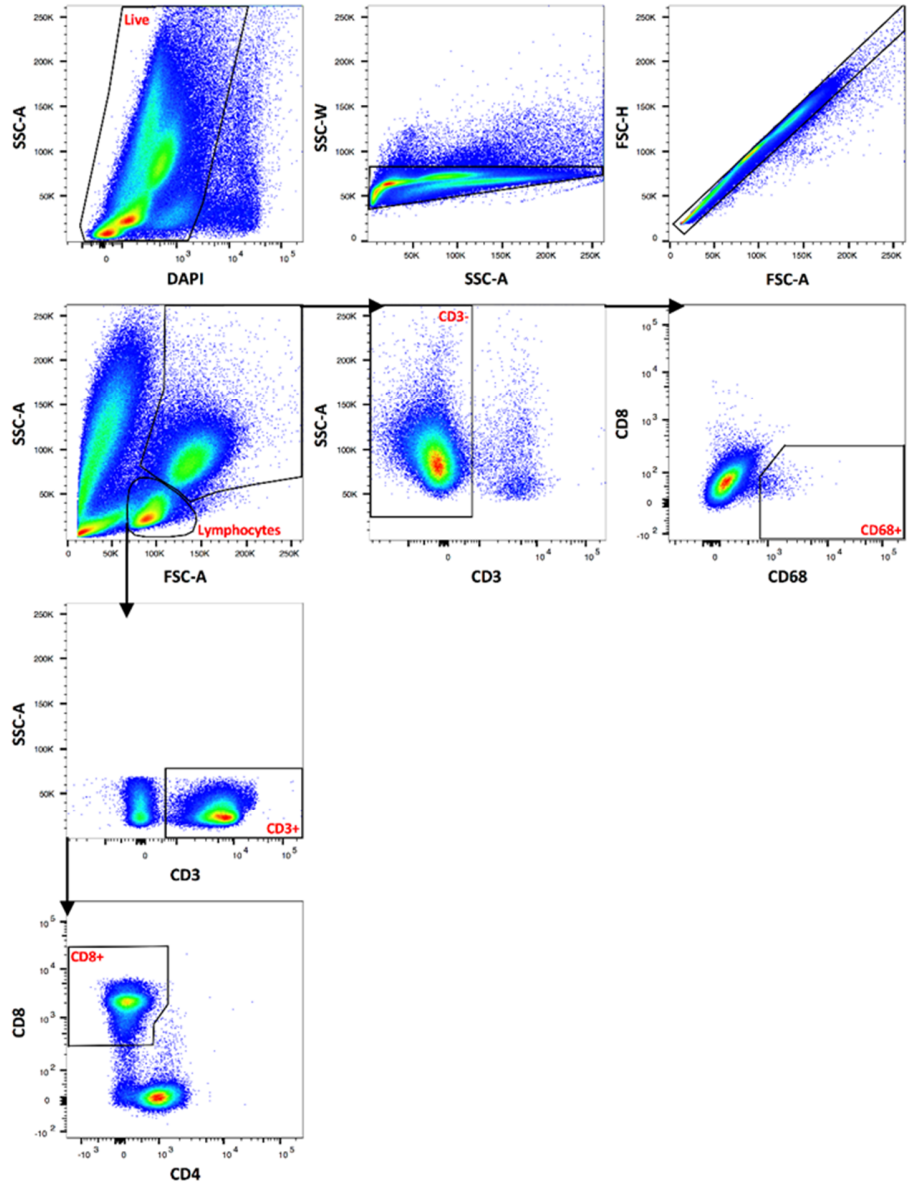
- Total bilirubin >4 mg/dL (not applicable for newborn) OR
- Alanine transaminase >2 times upper limit of normal for age

Supplemental Table 2. Analytes Tested in MagMix Panels

Luminex and Preferred Protein Names					
Luminex Name/ Abbreviation	**Preferred Protein Name (Abbreviation)**	**Luminex Name/ Abbreviation**	**Preferred Protein Name Abbreviation)**	**Luminex Name/ Abbreviation**	**Preferred Protein Name (Abbreviation)**
6CKine	C-C motif chemokine 21 (CCL21)	DKK1	Dickkopf-related protein 1 (DKK1)	HE4	WAP four-disulfide core domain protein 2
ACTH	Pro-opiomelanocortin (POMC)	ECM1	Extracellular matrix protein 1 (ECM1)	HGF	Hepatocyte Growth Factor
AFP	Alpha-fetoprotein (AFP)	EGF	Pro-epidermal growth factor (EGF)	HSP70	Heat shock 70 kDa protein 1A
Angiopoietin-2	Angiopoietin-2 (ANG-2)	ENA-78	C-X-C motif chemokine 5 (CXCL5)	I-309	C-C Motif Chemokine Ligand 1
Antithrombin III	Antithrombin-III (ATIII)	Endoglin	Endoglin	IFN-α2	Interferon alpha-2 (IFN-alpha-2)
BCA-1	C-X-C motif chemokine 13 (CXCL13)	Endothelin-1	Endothelin-1	IFN-y	Interferon Gamma (IFN-gamma)
β-HCG	Choriogonadotropin subunit beta 3	Eotaxin	Eotaxin	IL-1α	Interleukin-1 alpha (IL-1 alpha)
BMP-9	Growth/differentiation factor 2 (GDF-2)	Eotaxin-2	C-C motif chemokine 24	IL-1β	Interleukin-1 beta (IL-1 beta)
CA125	Mucin-16 (MUC-16)	Eotaxin-3	C-C motif chemokine 26	IL-1RA	Interleukin-1 receptor antagonist protein (IL-1ra)
CA15-3	Mucin-1 (MUC-1)	FGF-1	Fibroblast growth factor 1 (FGF-1)	IL-2	Interleukin-2 (IL-2)
CA19-9	Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 6	FGF-2	Fibroblast growth factor 2 (FGF-2)	IL-3	Interleukin-3 (IL-3)
CCL19/MIP-3β	C-C motif chemokine 19 (CCL19)	FGF-23	Fibroblast growth factor 23 (FGF-23)	IL-4	Interleukin-4 (IL-4)
CCL20/MIP-3α	C-C motif chemokine 20 (CCL20)	Flt-3L	Fms-related tyrosine kinase 3 ligand (Flt-3L)	IL-5	Interleukin-5 (IL-5)
CEA	Carcinoembryonic antigen	Follistatin	Follistatin (FS)	IL-6	Interleukin-6 (IL-6)
CFH	Complement factor H (CFH)	Fractalkine	Fractalkine	IL-7	Interleukin-7 (IL-7)
CTACK	C-C motif chemokine 27 (CCL27)	G-CSF	Granulocyte colony-stimulating factor (G-CSF)	IL-8	Interleukin-8 (IL-8)
CXCL11/I-TAC	C-X-C motif chemokine 11 (CXCL11)	GM-CSF	Granulocyte-macrophage colony-stimulating factor (GM-CSF)	IL-9	Interleukin-9 (IL-9)
CXCL6/GCP-2	C-X-C motif chemokine 6 (CXCL6)	Granzyme-B	Granzyme B	IL-10	Interleukin-10 (IL-10)
CXCL9/MIG	C-X-C motif chemokine 9 (CXCL9)	GRO	Growth-regulated alpha protein (GRO-alpha)	IL-11	Interleukin-11 (IL-11)
CYFRA21-1	CYFRA21-1	HB-EGF	Proheparin-binding EGF-like growth factor (HBEGF)	IL-12(p40)	Interleukin-12 subunit beta (IL-12B)
IL-12(p70)	Interleukin-12 subunit p70 (IL-12[p70])	MCP-4	C-C motif chemokine 13 (CCL13)	OPN	Osteopontin
IL-13	Interleukin-13 (IL-13)	M-CSF	Macrophage colony-stimulating factor 1 (M-CSF)	PLGF	Placenta growth factor (PlGF)
IL-15	Interleukin-15 (IL-15)	MDC	C-C motif chemokine 22 (CCL22)	Prolactin	Prolactin (PRL)
IL-16	Pro-interleukin-16 (IL-16)	MIF	Macrophage migration Inhibitory factor (MIF)	PTH	Prolactin (PRL)
IL-17A	Interleukin-17A (IL-17A)	MIP-1α	C-C motif chemokine 3 (CCL3)	RANTES	Parathyroid hormone (PTH)
IL-20	Interleukin-20 (IL-20)	MIP-1β	C-C motif chemokine 4 (CCL4)	sCD30	C-C motif chemokine 5
IL-21	Interleukin-21 (IL-21)	MIP-1δ	C-C motif chemokine 15 (CCL15)	SCD-40L	Tumor necrosis factor receptor superfamily member 8
IL-23	Interleukin-23 (IL-23)	MMP-1	Interstitial collagenase	SCF	CD40 ligand (CD40-L)
IL-28a	Interferon lambda-2	MMP-2	72 kDa type IV collagenase	SDF-1-α+β	c-kit ligand

IL-29/IFN-y1	Interferon lambda-1	MMP-3	Stromelysin-1 (SL-1)	sEGFR	Stromal cell-derived factor 1 alpha and beta (SDF-1-alpha and beta)
IL-33	Interleukin-33 (IL-33)	MMP-7	Matrilysin	sFAS	Epidermal growth factor receptor
Insulin	Insulin	MMP-8	Neutrophil collagenase	sFASl	Tumor necrosis factor receptor superfamily member 6 (FASLG receptor)
IP-10	C-X-C motif chemokine 10 (CXCL10)	MMP-9	Matrix metalloproteinase-9 (MMP-9)	sgp130	Tumor necrosis factor ligand superfamily member 6 (FasL)
Leptin	Leptin	MMP-10	Stromelysin-2	sIL-1RI	Interleukin-6 receptor subunit beta (IL-6R-beta)
LIF	Leukemia inhibitory factor (LIF)	MMP-12	Macrophage metalloelastase (MME)	sIL-1RII	Interleukin-1 receptor type 1 (IL-1R-1)
MCP-1	C-C motif chemokine 2 (CCL2)	MMP-13	Collagenase 3	sIL-2Ra	Interleukin-1 receptor type 2 (IL-1R-2)
MCP-2	C-C motif chemokine 8 (CCL8)	OC	Osteocalcin	sIL-4R	Interleukin-2 receptor subunit alpha (IL-2R subunit alpha)
MCP-3	C-C motif chemokine 7 (CCL7)	OPG	Tumor necrosis factor receptor superfamily member 11B	sIL-6R	Interleukin-4 receptor subunit alpha (IL-4R subunit alpha)
SOST	Interleukin-6 receptor (subunit alpha and beta) [IL-6R-(alpha+beta)]	TGFβ-1	Transforming growth factor beta-1 (TGF-beta-1)	TRAIL	Tumor necrosis factor ligand superfamily member 10 (TNFSF10)
sRAGE	Advanced glycosylation end product-specific receptor	TGFβ-2	Transforming growth factor beta-2 (TGF-beta-2)	TSLP	Thymic stromal lymphopoietin
sTNFRI	Tumor necrosis factor receptor superfamily member 1A (TNF-R1)	TGFβ-3	Transforming growth factor beta-3 (TGF-beta-3)	VEGF	Vascular endothelial growth factor A (VEGF-A)
sTNFRII	Tumor necrosis factor receptor superfamily member 1B (TNF-R2)	TIMP-1	Metalloproteinase inhibitor 1	VEGF-A	Vascular endothelial growth factor A (VEGF-A)
sVEGFR1	Vascular endothelial growth factor receptor 1 (VEGFR-1)	TIMP-2	Metalloproteinase inhibitor 2	VEGF-C	Vascular endothelial growth factor C (VEGF-C)
sVEGFR2	Vascular endothelial growth factor receptor 2 (VEGFR-2)	TNF-α	Tumor necrosis factor	VEGF-D	Vascular endothelial growth factor D (VEGF-D)
sVEGFR3	Vascular endothelial growth factor receptor 3 (VEGFR-3)	TNF-β	Lymphotoxin-alpha (LT-alpha)	Vitamin D BP	Vitamin D-binding protein (DBP)
TARC	C-C motif chemokine 17 (CCl17)	Total PSA	Total Prostate-specific antigen (Total PSA)	Vitronectin	Vitronectin (VN)
TGF-α	Transforming growth factor alpha (TGF-alpha)	TPO	Thyroid peroxidase (TPO)	XCL1/ Lymphotactin	Lymphotactin

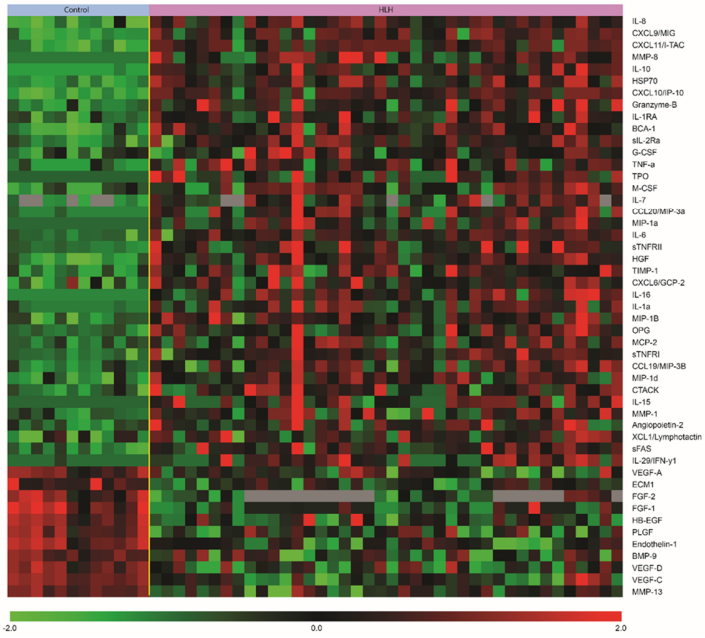
Supplemental Figures



Supplemental Figure 1. Peripheral blood flow sorting strategy

Peripheral blood mononuclear cells from patients with active HLH (pre-therapy) and severe sepsis were sorted for FACS in specific populations: $CD3^{+}8^{+}$ cytotoxic T cells and CD68+ monocytes. RNA was isolated from these cells and used for comparative gene expression studies (Figure 4).

Analytes	Training				Validation			
	Log Transformed Mean Concentration (pg/ml)		Fold Change	p-value	Log Transformed Mean Concentration (pg/ml)		Fold Change	p-value
	HLH	Control			HLH	Control		
IL-8	6.9 ± 1.4	1.7 ± 2.3	37.04	3.00E-07	6.7 ± 2.1	0.9 ± 1.2	55.56	8.34E-04
CXCL9/MIG	14.2 ± 2.2	8.6 ± 0.4	50.00	2.20E-06	13.3 ± 2.6	8.1 ± 1.1	37.04	6.88E-03
CXCL11/I-TAC	10.0 ± 2.1	4.1 ± 1.3	58.82	6.00E-07	9.8 ± 2.1	4.6 ± 1.0	35.71	9.62E-03
MMP-8	11.3 ± 2.9	7.0 ± 0.0	18.87	1.53E-03	12.2 ± 3.6	7.0 ± 0.0	35.71	2.25E-03
IL-10	7.6 ± 2.4	1.9 ± 0.4	50.00	5.70E-06	6.8 ± 2.5	1.8 ± 0.0	32.26	3.34E-02
IL-8 (2)	5.8 ± 2.5	0.5 ± 0.0	40.00	2.96E-05	5.2 ± 3.0	0.5 ± 0.0	27.03	3.18E-02
HSP70	19.0 ± 2.4	14.4 ± 0.8	25.64	1.10E-04	19.2 ± 2.1	14.7 ± 0.6	22.22	8.66E-05
CXCL10/IP-10	12.7 ± 1.1	8.3 ± 0.5	21.28	1.00E-07	12.3 ± 1.6	7.9 ± 0.7	20.41	6.90E-06
Granzyme-B	6.2 ± 3.3	1.5 ± 0.9	25.64	2.12E-03	5.9 ± 2.7	1.7 ± 1.6	18.18	2.16E-03
IL-1RA	7.7 ± 2.4	4.4 ± 1.2	10.20	3.03E-03	7.6 ± 2.8	3.9 ± 1.8	12.99	9.57E-03
BCA-1	8.1 ± 1.2	5.4 ± 1.0	6.67	1.83E-05	8.3 ± 1.5	4.7 ± 0.8	11.49	1.12E-05
sIL-2Ra	13.4 ± 1.5	9.2 ± 1.9	18.52	4.70E-06	13.2 ± 2.5	10.0 ± 0.9	9.09	5.91E-03
G-CSF	6.0 ± 1.5	4.4 ± 1.3	2.94	2.88E-02	6.6 ± 3.0	3.5 ± 1.5	8.33	1.47E-03
TNF-a (2)	5.4 ± 1.6	2.3 ± 1.1	8.33	5.13E-04	5.4 ± 1.4	2.4 ± 1.3	7.69	1.82E-03
TPO	8.6 ± 1.7	6.2 ± 1.0	5.26	2.49E-03	8.9 ± 1.9	5.9 ± 0.7	7.69	9.49E-04
M-CSF	9.5 ± 2.2	6.7 ± 0.0	7.14	4.95E-03	9.5 ± 3.0	6.7 ± 0.0	7.14	3.35E-02
IL-7	2.5 ± 1.7	0.4 ± 1.2	4.35	1.09E-02	2.6 ± 1.8	-0.2 ± 1.0	6.67	1.90E-06
CCL20/MIP-3a	6.0 ± 1.8	2.8 ± 0.4	9.09	2.57E-03	5.9 ± 2.0	3.3 ± 0.4	6.25	3.98E-02
MIP-1a	4.3 ± 1.8	1.6 ± 0.0	6.67	1.01E-03	4.4 ± 1.8	1.8 ± 0.3	5.88	2.26E-03
IL-6	4.2 ± 2.1	1.5 ± 0.0	6.67	5.08E-03	4.0 ± 2.4	1.5 ± 0.0	5.88	1.88E-03
sTNFRII	15.5 ± 1.1	11.4 ± 2.4	18.18	1.50E-06	14.7 ± 2.6	12.2 ± 0.5	5.56	3.08E-02
HGF	10.1 ± 2.2	6.8 ± 0.3	9.09	1.50E-03	9.8 ± 2.1	7.3 ± 0.3	5.56	4.28E-05
TNF-a (3)	3.4 ± 1.8	0.4 ± 0.9	7.69	1.30E-03	3.1 ± 1.3	0.6 ± 1.0	5.56	2.48E-03
TIMP-1	17.9 ± 0.9	15.1 ± 0.7	7.14	4.00E-07	17.7 ± 1.2	15.2 ± 0.8	5.56	6.57E-05
CXCL6/GCP-2	6.6 ± 1.5	3.9 ± 1.0	6.25	4.44E-04	6.4 ± 1.9	4.1 ± 0.7	5.00	9.43E-05
IL-16	8.0 ± 1.1	5.7 ± 1.3	5.00	1.30E-04	8.2 ± 1.8	6.0 ± 2.0	4.55	7.91E-04
IL-8 (3)	6.1 ± 2.1	3.0 ± 0.0	9.09	1.84E-03	5.1 ± 2.1	3.0 ± 0.0	4.35	2.20E-06
IL-1a	5.7 ± 1.5	2.9 ± 0.0	6.67	1.84E-04	5.1 ± 1.3	2.9 ± 0.0	4.35	5.58E-03
TNF-a	4.9 ± 1.3	2.9 ± 0.1	4.00	1.10E-03	5.2 ± 1.7	3.0 ± 0.4	4.35	6.05E-03
MIP-1B	5.8 ± 0.9	4.4 ± 0.4	2.70	1.25E-03	6.0 ± 1.0	3.9 ± 1.2	4.35	2.55E-04
OPG	9.9 ± 2.1	7.0 ± 0.7	7.14	2.78E-03	9.2 ± 1.8	7.2 ± 0.3	4.17	1.35E-02
MIP-1a (2)	4.8 ± 2.2	2.5 ± 0.0	5.00	2.20E-02	4.6 ± 2.1	2.5 ± 0.0	4.17	3.35E-02
MCP-2	5.1 ± 1.5	3.4 ± 0.5	3.23	1.40E-02	5.3 ± 1.9	3.3 ± 0.6	4.17	1.69E-02
sTNFRI	12.1 ± 1.1	8.8 ± 1.7	10.10	6.10E-06	11.5 ± 1.8	9.5 ± 0.4	4.00	1.37E-02
CCL19/MIP-3B	8.5 ± 1.5	6.6 ± 0.7	3.70	7.89E-03	8.1 ± 1.6	6.1 ± 0.6	3.85	8.51E-03
MIP-1d	12.1 ± 1.0	9.9 ± 1.5	4.55	2.84E-04	12.1 ± 1.0	10.2 ± 0.9	3.70	2.41E-04
CTACK	9.4 ± 1.0	8.1 ± 0.9	2.44	1.02E-02	9.7 ± 1.1	8.0 ± 0.4	3.23	1.40E-03
IL-15	3.2 ± 1.4	1.3 ± 0.0	3.85	2.51E-03	3.0 ± 1.8	1.3 ± 0.0	3.13	1.99E-02
MMP-1	10.0 ± 1.6	8.3 ± 0.5	3.23	2.00E-02	10.3 ± 1.4	8.6 ± 0.7	3.03	1.49E-02
Angiopoietin-2	12.7 ± 1.5	10.7 ± 0.7	4.17	3.65E-03	12.4 ± 1.6	10.9 ± 0.6	2.70	4.66E-02
XCL1/Lymphotactin	6.8 ± 1.0	5.7 ± 1.2	2.17	2.79E-02	6.4 ± 1.1	5.1 ± 1.4	2.44	3.04E-02
sFAS	11.8 ± 0.9	10.0 ± 0.4	3.33	1.10E-04	11.6 ± 1.0	10.4 ± 0.4	2.27	1.18E-02
IL-29/IFN-y1	7.7 ± 1.0	6.5 ± 0.0	2.22	8.65E-03	7.7 ± 0.9	6.6 ± 0.2	2.08	1.61E-02
VEGF-A	6.4 ± 1.7	8.2 ± 0.8	-3.52	1.70E-02	7.3 ± 1.2	8.7 ± 0.9	-2.62	1.74E-02
ECM1	20.1 ± 0.9	22.1 ± 2.3	-4.05	3.31E-03	20.0 ± 1.0	21.4 ± 0.9	-2.62	8.85E-05
FGF-2 (3)	6.9 ± 0.7	7.7 ± 0.6	-1.79	2.47E-02	6.4 ± 0.5	8.2 ± 0.7	-3.47	2.34E-02
FGF-1	4.4 ± 0.7	5.5 ± 0.6	-2.15	2.65E-03	4.4 ± 0.5	6.2 ± 0.5	-3.57	1.08E-04
HB-EGF	4.0 ± 1.0	5.5 ± 0.8	-2.74	4.04E-03	4.2 ± 1.3	6.1 ± 0.5	-3.81	1.08E-02
PLGF	3.3 ± 1.5	4.9 ± 0.6	-3.03	2.15E-02	3.1 ± 1.2	5.1 ± 1.1	-4.05	1.42E-03
Endothelin-1	3.8 ± 1.3	5.7 ± 0.6	-3.63	2.05E-03	3.8 ± 1.1	6.1 ± 0.8	-4.89	1.00E-07
BMP-9	5.0 ± 2.2	7.2 ± 0.7	-4.61	2.57E-02	5.3 ± 2.4	8.0 ± 0.8	-6.40	1.38E-02
VEGF-D	5.6 ± 1.9	7.6 ± 0.7	-3.93	2.25E-02	5.2 ± 1.7	8.0 ± 1.2	-6.66	1.18E-03
VEGF-C	6.2 ± 2.0	8.5 ± 0.4	-4.74	1.27E-02	5.5 ± 1.6	9.0 ± 0.6	-10.72	4.82E-05
MMP-13	8.2 ± 1.7	10.2 ± 0.6	-4.06	7.55E-03	6.4 ± 3.3	10.6 ± 0.9	-18.22	5.79E-03

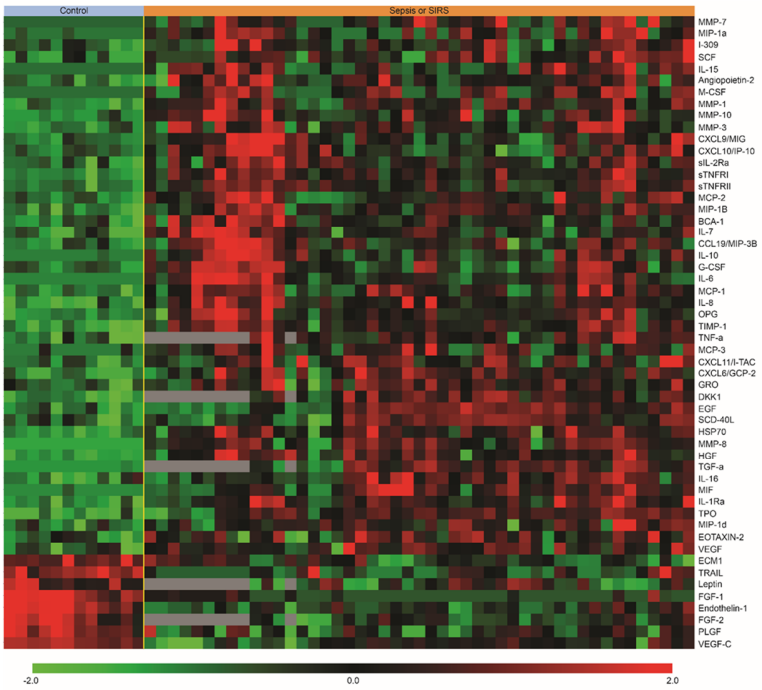


Supplemental Figure 2A. Plasma inflammatory proteins: HLH versus control

(left) Heatmap demonstrates relative concentration of analytes with significant differential expression using supervised comparison of HLH and non-inflammatory controls.

(right) Values for analytes with differential expression between HLH and non-inflammatory control datasets are displayed for discovery and validation cohorts.

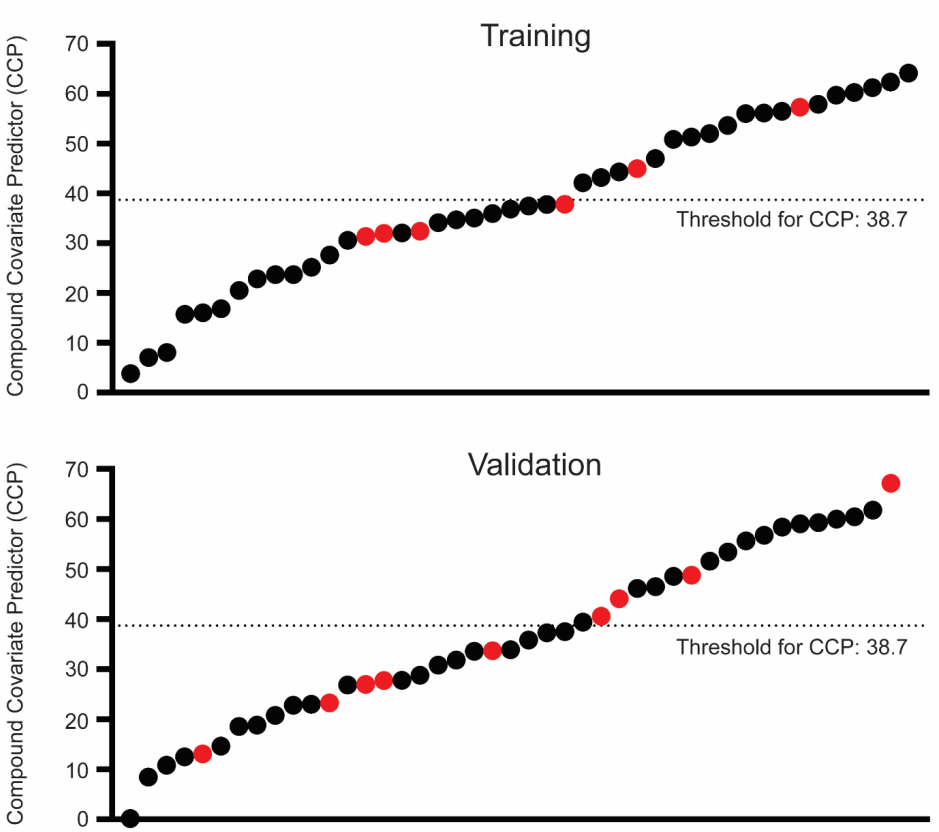
Analytes	Training				Validation			
	Log Transformed Mean Concentration (pg/ml)		Fold	p-value	Log Transformed Mean Concentration (pg/ml)		Fold	p-value
	Sepsis or SIRS	CTL	Change		Sepsis or SIRS	CTL	Change	
IL-6 (2)	5.9 ± 3.2	-1.6 ± 0.0	172.41	1.41E-05	3.9 ± 0.5	-1.6 ± 0.0	227.27	1.42E-04
MMP-8	14.5 ± 3.2	7.0 ± 0.0	172.41	7.30E-06	3.5 ± 0.5	7.0 ± 0.0	166.67	8.00E-06
IL-8	8.1 ± 3.1	1.7 ± 2.3	83.33	6.54E-05	6.5 ± 0.4	0.9 ± 1.2	83.33	3.00E-07
IL-8 (2)	6.8 ± 4.1	0.5 ± 0.0	76.92	9.55E-04	7.3 ± 2.3	0.5 ± 0.0	58.82	1.44E-04
IL-6	7.2 ± 3.5	1.5 ± 0.0	52.63	4.90E-04	17.9 ± 1.1	1.5 ± 0.0	38.46	1.81E-03
IL-1RA	9.3 ± 2.4	4.4 ± 1.2	29.41	6.50E-05	14.4 ± 3.3	3.9 ± 1.8	27.78	9.57E-05
G-CSF	8.2 ± 3.4	4.4 ± 1.3	13.89	1.37E-02	11.9 ± 1.1	3.5 ± 1.5	23.26	1.40E-02
HSP70	18.4 ± 2.1	14.4 ± 0.8	16.39	1.24E-04	9.1 ± 1.4	14.7 ± 0.6	14.08	1.48E-04
IL-10	6.0 ± 2.5	1.9 ± 0.4	16.95	6.41E-04	11.0 ± 1.0	1.8 ± 0.0	13.70	4.38E-03
IL-8 (3)	7.2 ± 2.9	3.0 ± 0.0	19.61	1.60E-03	6.1 ± 0.8	3.0 ± 0.0	13.51	1.62E-03
MIF	12.6 ± 3.2	7.3 ± 0.0	40.00	3.80E-04	4.1 ± 1.5	7.6 ± 0.6	12.99	7.90E-04
EGF	8.0 ± 2.5	4.3 ± 1.5	13.33	1.94E-03	6.1 ± 1.5	4.6 ± 1.4	12.35	2.15E-03
TPO	9.3 ± 1.2	6.2 ± 1.0	8.33	2.70E-06	8.7 ± 2.4	5.9 ± 0.7	9.09	9.60E-06
CXCL6/GCP-2	7.1 ± 1.7	3.9 ± 1.0	9.09	1.81E-04	10.3 ± 1.6	4.1 ± 0.7	9.09	2.98E-04
CXCL9/MIG	11.4 ± 1.9	8.6 ± 0.4	7.14	9.63E-04	6.3 ± 4.1	8.1 ± 1.1	9.09	1.54E-02
TGF-a (2)	4.2 ± 1.5	1.1 ± 0.1	8.33	7.46E-05	6.4 ± 3.3	1.0 ± 0.0	8.33	4.21E-06
HGF	11.1 ± 1.7	6.8 ± 0.3	18.87	2.70E-06	18.5 ± 2.1	7.3 ± 0.3	8.33	1.38E-04
OPG	10.2 ± 1.8	7.0 ± 0.7	9.09	3.80E-04	2.9 ± 1.2	7.2 ± 0.3	7.14	9.00E-04
TIMP-1	18.0 ± 1.1	15.1 ± 0.7	7.59	1.80E-06	14.2 ± 1.1	15.2 ± 0.8	6.67	3.70E-06
MMP-10	10.4 ± 1.6	8.3 ± 0.7	4.35	4.12E-03	10.7 ± 1.0	8.0 ± 0.2	6.67	2.77E-04
M-CSF	9.3 ± 2.2	6.7 ± 0.0	6.25	7.81E-03	10.7 ± 1.6	6.7 ± 0.0	6.67	6.06E-03
IL-8 (4)	7.0 ± 3.0	3.4 ± 0.8	12.20	8.38E-03	7.3 ± 1.8	4.0 ± 0.8	6.67	1.88E-02
MCP-1	10.3 ± 1.5	7.4 ± 1.1	7.69	1.87E-04	3.7 ± 1.3	7.9 ± 0.7	6.25	1.85E-03
IL-16	9.0 ± 2.0	5.7 ± 1.3	10.00	8.38E-04	8.3 ± 1.2	6.0 ± 2.0	6.25	4.86E-03
IL-6 (3)	5.3 ± 2.3	3.1 ± 0.0	4.76	2.80E-02	8.4 ± 1.1	3.1 ± 0.0	6.25	5.45E-02
CXCL11/I-TAC	7.4 ± 2.2	4.1 ± 1.3	10.31	1.14E-03	4.7 ± 1.2	4.6 ± 1.0	5.88	1.42E-02
sTNFRI	11.9 ± 1.2	8.8 ± 1.7	8.33	2.64E-05	3.8 ± 1.1	9.5 ± 0.4	5.26	9.50E-06
MMP-1	10.7 ± 1.7	8.3 ± 0.5	5.56	1.25E-03	4.7 ± 1.3	8.8 ± 0.7	5.26	1.25E-05
MMP-3	14.2 ± 1.5	12.1 ± 0.7	4.17	3.21E-03	11.2 ± 2.4	11.9 ± 0.4	5.26	9.76E-04
IL-7	2.9 ± 1.4	0.4 ± 1.2	5.56	6.84E-04	10.0 ± 1.8	-0.2 ± 1.0	5.26	1.75E-03
IP-10	10.2 ± 1.7	8.3 ± 0.5	3.70	1.34E-02	14.3 ± 1.6	7.9 ± 0.7	5.00	2.42E-02
TNF-a (2)	5.0 ± 1.2	2.3 ± 1.1	6.25	6.46E-05	5.6 ± 1.0	2.4 ± 1.3	4.76	5.06E-04
MIP-1B	6.2 ± 0.8	4.4 ± 0.4	3.33	3.84E-05	13.0 ± 1.4	3.9 ± 1.2	4.55	1.46E-05
VEGF	8.3 ± 1.1	6.8 ± 1.9	2.94	1.35E-02	6.7 ± 2.5	6.2 ± 1.6	4.55	5.01E-04
SCF	5.0 ± 2.0	2.5 ± 1.2	5.56	7.89E-03	2.9 ± 1.1	2.5 ± 1.2	4.55	6.14E-04
GRO	10.3 ± 1.7	7.1 ± 2.6	9.09	1.28E-03	2.2 ± 1.6	7.9 ± 0.6	4.55	2.20E-02
CCL19/MIP-3B	7.9 ± 1.4	6.6 ± 0.7	2.56	2.87E-02	19.4 ± 1.4	6.1 ± 0.6	4.35	4.08E-04
MCP-2	5.0 ± 1.4	3.4 ± 0.5	3.13	1.05E-02	6.7 ± 3.7	3.3 ± 0.6	4.35	3.40E-03
SCD-40L	11.1 ± 2.1	8.0 ± 1.5	8.33	2.16E-03	10.6 ± 1.8	9.1 ± 1.5	4.35	4.12E-02
Angiopoietin-2	12.6 ± 1.5	10.7 ± 0.7	3.85	3.76E-03	8.2 ± 2.5	10.9 ± 0.6	4.17	1.55E-03
sIL-2Ra	12.2 ± 1.4	9.2 ± 1.9	7.69	1.87E-04	12.0 ± 1.4	10.0 ± 0.9	4.17	2.28E-03
BCA-1	7.1 ± 1.2	5.4 ± 1.0	3.23	4.03E-03	6.8 ± 1.5	4.7 ± 0.8	4.17	2.77E-03
sTNFRII	14.6 ± 1.1	11.4 ± 2.4	9.09	5.38E-05	5.4 ± 1.6	12.2 ± 0.5	4.00	2.17E-04
MIP-1a	3.7 ± 1.7	1.6 ± 0.0	4.35	5.99E-03	5.5 ± 2.9	1.8 ± 0.3	4.00	8.11E-03
DKK1	10.7 ± 0.8	8.0 ± 0.4	6.25	1.00E-07	8.6 ± 1.9	8.8 ± 0.2	3.70	2.48E-04
TNF-a (3)	2.7 ± 1.2	0.4 ± 0.9	5.00	2.37E-04	2.4 ± 1.3	0.6 ± 1.0	3.45	5.29E-03
MCP-3	6.1 ± 2.0	3.5 ± 1.1	5.88	7.08E-03	9.4 ± 2.2	3.5 ± 1.1	3.33	3.16E-02
MIP-1B (2)	5.4 ± 1.2	4.0 ± 0.5	2.70	7.35E-03	13.7 ± 1.0	4.0 ± 0.9	3.13	1.54E-03
IL-15	2.9 ± 1.6	1.3 ± 0.0	3.03	1.83E-02	11.5 ± 1.0	1.3 ± 0.0	3.13	1.73E-03
EOTAXIN-2	8.9 ± 1.6	6.9 ± 1.1	4.17	6.65E-03	3.8 ± 1.7	7.9 ± 1.0	2.86	4.18E-02
MIP-1d	11.8 ± 1.4	9.9 ± 1.5	3.57	7.50E-03	8.1 ± 4.1	10.2 ± 0.9	2.44	7.03E-03
MMP-7	13.7 ± 1.3	12.5 ± 0.0	2.33	2.69E-02	7.2 ± 2.3	12.5 ± 0.0	2.38	6.61E-03
I-309	2.9 ± 1.1	1.7 ± 0.5	2.22	2.07E-02	11.3 ± 2.9	1.8 ± 0.5	1.75	2.06E-02
FGF-2 (3)	6.8 ± 0.6	7.7 ± 0.6	0.53	3.53E-03	6.7 ± 2.6	8.2 ± 0.7	0.33	1.00E-07
TRAIL	3.6 ± 1.0	5.8 ± 0.5	0.21	1.34E-05	10.3 ± 2.4	5.7 ± 0.7	0.28	5.13E-04
ECM1	19.0 ± 1.5	22.1 ± 2.3	0.11	4.24E-04	2.6 ± 0.8	21.4 ± 0.9	0.25	1.78E-03
TRAIL (2)	3.5 ± 1.2	5.8 ± 0.7	0.21	2.82E-04	10.1 ± 2.2	6.0 ± 0.4	0.21	3.29E-04
PLGF	3.2 ± 1.2	4.9 ± 0.6	0.30	2.22E-03	10.2 ± 2.3	5.1 ± 1.1	0.21	1.75E-04
FGF-1	4.0 ± 0.4	5.5 ± 0.6	0.35	2.00E-07	5.2 ± 1.8	6.2 ± 0.5	0.20	1.00E-07
Endothelin-1	4.0 ± 0.8	5.7 ± 0.6	0.32	4.83E-05	11.2 ± 2.3	6.1 ± 0.8	0.16	1.00E-07
Leptin (3)	10.4 ± 2.1	12.6 ± 1.6	0.22	2.89E-02	9.4 ± 1.6	13.0 ± 1.7	0.15	1.97E-02
VEGF-C	6.4 ± 1.5	8.5 ± 0.4	0.23	2.14E-03	5.7 ± 3.1	9.0 ± 0.6	0.13	8.26E-05



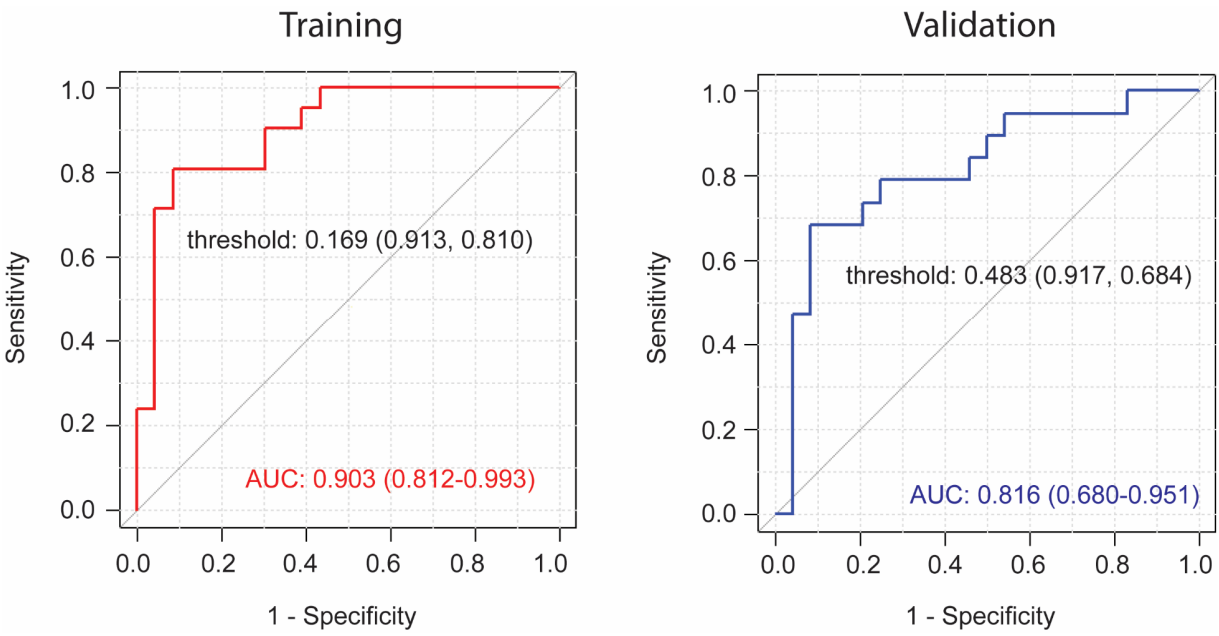
Supplemental Figure 2B. Plasma inflammatory proteins: Sepsis/SIRS versus control

(left) Heatmap demonstrates relative concentration of analytes with significant differential expression using supervised comparison of SIRS/sepsis and non-inflammatory controls.

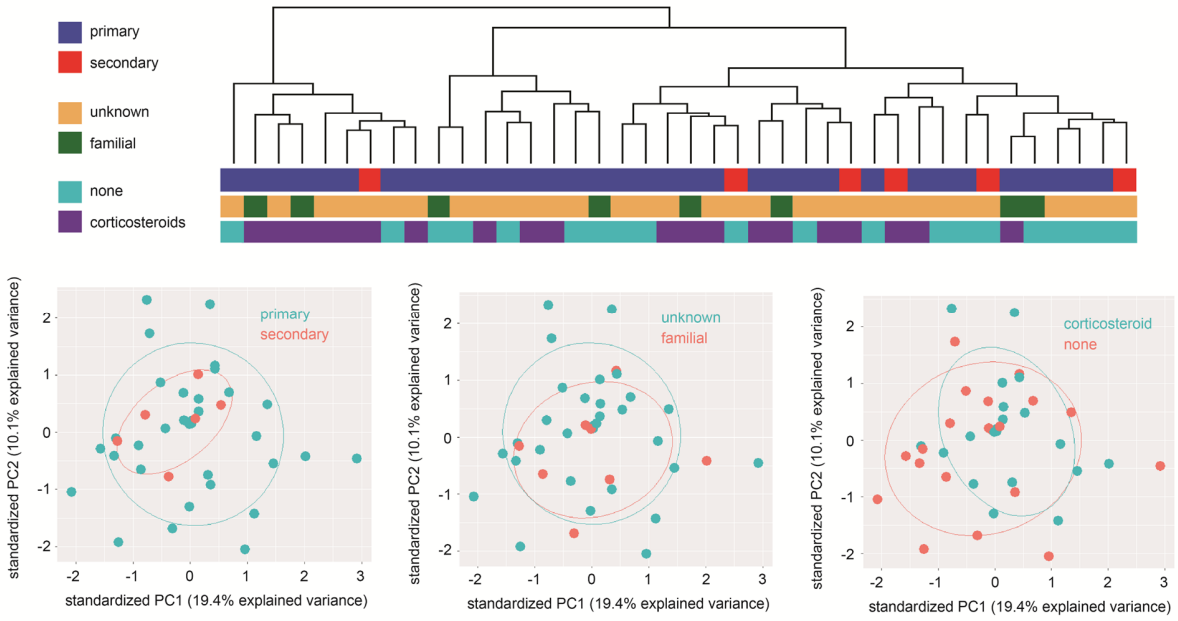
(right) Values for analytes with differential expression between HLH and non-inflammatory control datasets are displayed for discovery and validation cohorts.



Supplemental Figure 3. Compound Covariate Predictor Performance. Scatterplots of the compound covariate predictor scores for each sample in the classifier of the training and validation sets are presented. The prediction score is defined by the inner sum of the weights (w_i) and log intensity (x_i) of each significant protein. The weight for each protein is determined using the t statistic of differential expression in the training set and the threshold is the midpoint means of the two classes ($C_t = (C_1+C_2)/2$). A sample is classified as HLH if the sum is greater than the threshold ($\sum w_i x_i >$ threshold). Incorrectly classified samples are shown in red.

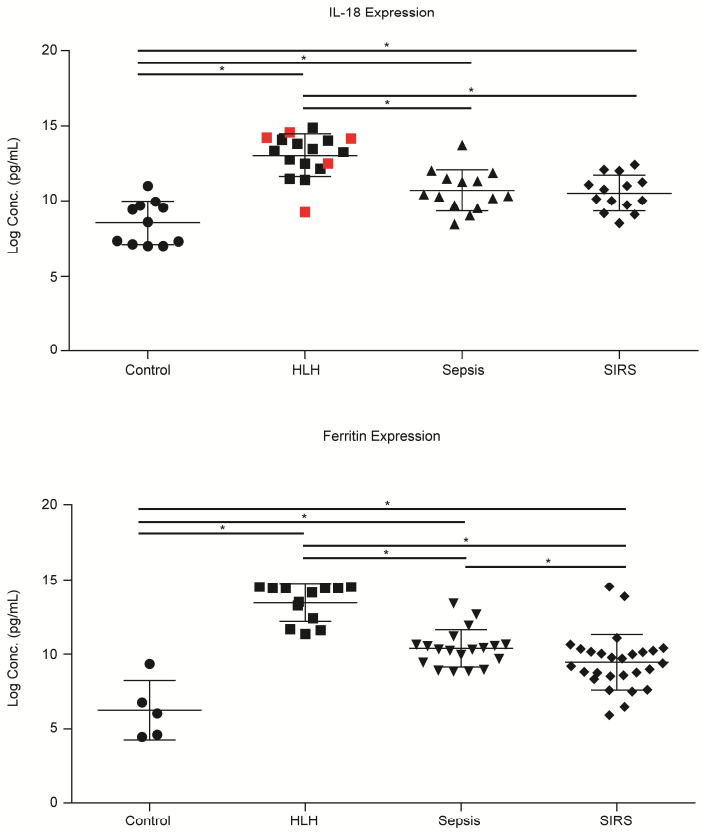


Supplemental Figure 4. Performance of CXCL9/IL6 Ratio as a Classifier. Receiver operating curves of the CXCL9/IL6 ratio in training and validation sets for diagnostic accuracy of HLH or sepsis/SIRS. Receiver operating curves of the CXCL9/IL6 ratio in discovery and validation sets for diagnostic accuracy of HLH or sepsis/SIRS. The ratio thresholds were chosen to derive the best specificity and sensitivity based on the Youden index (the numbers in parentheses following "threshold"). AUC denotes the area under then ROC and the numbers in parenthesis are 95% CI.



Supplemental Figure 5. Unsupervised Clustering of HLH Plasma Samples

Unsupervised clustering (A) and principal component analysis (PCA) (B) demonstrate lack of grouping of HLH specimens based on classes: primary (not autoimmune or cancer) vs secondary (associated with autoimmune disease or cancer); familial (proven HLH-associated genetic variants) vs unproven/unknown genetic associations; or no pretreatment with corticosteroids vs corticosteroid pretreatment prior to plasma collection. No statistical differences were noted between these groups, though it is possible differences could be identified with a larger dataset.



Supplemental Figure 6. IL-18 and ferritin: HLH versus sepsis and SIRS. Plots demonstrate IL-18 (top) and ferritin (bottom) concentrations for control, HLH, sepsis and SIRS groups. Bars with (*) indicate groups with statistically significant differential expression. Red indicates cases of secondary HLH.

Supplemental Files. Subject Details

Supplemental File 1A. HLH Subjects

Supplemental File 1B. SIRS/Sepsis Subjects

Attached as an excel file with separate tabs for Files 1A and 1B.