

Supplementary Appendix

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Supplementary Result

Downregulation of Mitochondrial Function-related Genes is Observed in Sepsis

We performed gene expression profiling in the first 29 patients recruited into the study. We used a commercially available metabolic/mitochondria focused biomarker panel (NanoString® Technologies Inc). Ten healthy controls were also included in the gene-expression profiling experiments. Given the potential for selection bias, we compared the baseline characteristics between these selected patients against the overall cohort ($n = 67$). Reassuringly, we found that there were no differences between these 29 patients and all other patients in the cohort in terms of age, gender, comorbidities, source of infections, microbiological results, clinical course, outcomes and laboratory findings (Table S2).

Impaired Mitochondrial Function is Observed in Uncomplicated Infection and Sepsis

Cellular metabolism was measured on the entire cohort (67 patients and 20 controls). This measurement confirmed a strong trend towards impaired mitochondrial functions across all patients. Notably, the degree of impairment was greater in the sepsis group compared to the uncomplicated infection group – a finding similar to that of the first 29 patients (Figure S1 A, 1B, 1C, 1D). However, no difference in glycolysis was found between groups as we found in the initial cohort (Figure S1 E).

**Table S1. List of Genes on NanoString's
Mitochondrial Biogenesis and Function Panel**

AIFM2	MIPEP	SLC25A30	Housekeeping
AIP	MPV17	SLC25A31	ABCF1
ATP5F1D	MSTO1	SLC25A37	GUSB
ATP5F1E	MTX2	SLC25A4	HPRT1
ATP5I	NEFL	SLC25A5	LDHA
ATP5L	NRF1	SOD1	POLR1B
ATP5O	OPA1	STARD3	RPLP0
BAK1	PMAIP1	TAZ	
BBC3	PPRC1	TIMM10	
BCL2	RHOT1	TIMM10B	
BCL2L1	RHOT2	TIMM17A	
BID	SH3GLB1	TIMM17B	
BNIP3	SLC25A1	TIMM22	
CDKN2A	SLC25A10	TIMM23	
COX10	SLC25A12	TIMM44	
COX18	SLC25A13	TIMM50	
CPT1B	SLC25A14	TIMM8A	
CPT2	SLC25A15	TIMM8B	
CYCS	SLC25A16	TIMM9	
DNM1L	SLC25A17	TOMM20	
FIS1	SLC25A19	TOMM22	
GRPEL1	SLC25A2	TOMM34	
IDH2	SLC25A20	TOMM40	
IMMP1L	SLC25A21	TOMM40L	
IMMP2L	SLC25A22	TP53	
IMMT	SLC25A23	TSPO	
LRPPRC	SLC25A24	UCP1	
MFN1	SLC25A25	UCP2	
MFN2	SLC25A27	UCP3	
MINOS1	SLC25A3	UXT	

Note: Two housekeeping genes, GUSB and RPLP0, were used to normalize the count. Another 4 housekeeping genes from this TagSet were excluded from the analyses as their expressions were found to be unstable between groups (data not shown).

Table S2. Demographic and Clinical Characteristic of Overall Subjects vs Subset of Subjects for Gene Expression Analysis

Characteristics	Overall	Subset	P value
N (%)	67 (100)	29 (43)	
Age – yr	64.0 ± 16.76	62.6 ± 20.85	0.7248
Male sex – no. (total no., %)	39 (58)	17 (59)	0.9277
SOFA score ≥2 (%)	40 (59.7)	15 (51.7)	0.4692
Source of infection			
Respiratory tract (%)	32 (47.8)	10 (34.5)	0.2302
Urinary tract (%)	16 (23.9)	6 (20.7)	0.7334
Abdominal, liver and biliary tract (%)	10 (14.9)	7 (24.1)	0.2805
Skin and soft tissue (%)	9 (13.4)	6 (20.7)	0.3608
Cardiovascular (%)	1 (1.5)	1 (3.4)	0.5507
Bone and joint (%)	2 (3.0)	1 (3.4)	0.9180
Unknown	3 (4.5)	0 (0)	0.2482
Comorbidities			
Cardiovascular disease (%)	45 (67.2)	21 (72.4)	0.6156
Respiratory disease (%)	20 (29.9)	4 (13.8)	0.0963
Diabetes mellitus (%)	21 (31.3)	10 (34.5)	0.7593
Malignancy (%)	17 (25.4)	3 (10.3)	0.0961
Chronic kidney disease (%)	9 (13.4)	4 (13.8)	0.9582
Septic shock (%)	11 (16.4)	4 (13.8)	0.7485
ICU admission (%)	9 (13.4)	4 (13.8)	0.9582
Hospital readmission – 28 day (%)	5/64 (7.8)	4/28 (14)	0.3575
Length of stay (day)	7 (0-106)	7 (0-106)	0.9066
In-hospital mortality (%)	3 (4.5)	1 (3.4)	0.8054
Improving SOFA score on 3-5 days (%)	23/26 (88)	6/8 (75)	0.3758
Leukocyte count (x10 ⁹ /mm ³)	13.3 (1.0-37.7)	16.0 ± 7.24	0.1533
Neutrophil count (x10 ⁹ /mm ³)	10.4 (0.0-36.1)	13.4 ± 6.90	0.1378
Lymphocyte count (x10 ⁹ /mm ³)	1.1 (0.2-5.1)	1.2 (0.3-4.2)	0.6235
Monocyte count (x10 ⁹ /mm ³)	0.9 (0.0-4.0)	0.9 (0.2-4.0)	0.1725
CRP (mg/L)	102 (3-390)	105 (3-319)	0.6268
Lactate (mmol/L)	1.6 (0.4-6.5), n = 52	1.6 (0.7-6.5), n = 21	0.96378
Procalcitonin (ng/mL)	2.0 (0.1-212.3), n = 15	3.1 (0.18-212.3), n = 6	0.7191
Positive culture			
From source of infection (%)	31/61 (51)	10/25 (40)	0.3566
From blood (%)	19/53 (34)	9/25 (36)	0.8633

Table S3. Top 20 Enriched Pathways in Sepsis Relative to Uncomplicated Infection

	Pathway	FDR	Ratio
1	Epithelial cell apoptosis in COPD	2.78E-03	0.063
2	Apoptosis and survival - Regulation of apoptosis by mitochondrial proteins	2.78E-03	0.061
3	Role of proinflammatory cytokines in activation of p53 in major depressive disorder	2.78E-03	0.043
4	Regulation of mast cell survival in asthma	2.78E-03	0.043
5	Suppression of p53 signaling in multiple myeloma	2.78E-03	0.040
6	Effect of <i>H. pylori</i> infection on apoptosis in gastric epithelial cells	2.78E-03	0.038
7	Immune response - IL-3 signaling via JAK/STAT, p38, JNK and NF- κ B	7.37E-03	0.022
8	Immune response - IL-3 signaling via ERK and PI3K	7.76E-03	0.020
9	Aminoglycoside- and cisplatin-induced hair cell death	8.31E-03	0.018
10	Dual role of p53 in transcription deregulation in Huntington's Disease	2.48E-02	0.083
11	Anti-apoptotic pathways in endoplasmic reticulum stress response in multiple myeloma	2.48E-02	0.053
12	Inhibition of RUNX3 signaling in gastric cancer	2.48E-02	0.045
13	Immune response - IL-15 signaling via JAK-STAT cascade	2.48E-02	0.045
14	Inhibition of GSK3 beta by lithium in major depressive disorder	2.48E-02	0.045
15	Action of GSK3 beta in bipolar disorder	2.48E-02	0.043
16	Role of alpha-V/ beta-6 integrin in colorectal cancer	2.48E-02	0.043
17	Dual role of TGF-beta 1 in HCC	2.48E-02	0.042
18	Immune response - IL-2 signaling via JAK/ STAT	2.48E-02	0.040
19	CREB1-dependent transcription deregulation in Huntington's Disease	2.48E-02	0.038
20	Hedgehog signaling in prostate cancer	2.48E-02	0.038

COPD chronic obstructive pulmonary disease; CREB1 cAMP (cyclic adenosine monophosphate) responsive element binding protein 1; TGF tumor growth factor.

Ratio: ratio between number of DEGs involved relative to the total number of network objects in the pathway.

Table S4. Linear Regression Results – Association Between Infection Severity (Categorized with SOFA Score) and Metabolic Parameters

Parameters	Regression	Regression	Δ Regression	P value	P Value
	Coefficient	Coefficient	Coefficient		
	Model	Model	(%)	Model	Model
	without Age	with Age		without Age	with Age
Mitochondrial Function					
Basal respiration	0.025	0.023	8.70	0.000	0.002
Maximal respiration	0.075	0.067	11.94	0.002	0.007
Spare respiratory capacity	0.05	0.044	13.64	0.007	0.022
ATP production	0.024	0.022	9.09	0.001	0.004
ECAR	0.003	0.003	0.00	0.214	0.332
Oxidative Stress					
DCFDA	-8.606	-8.893	-3.23	0.110	0.117
MitoSOX	-1.483	-0.999	48.45	0.017	0.113

Δ difference between the model without and with additional independent variable (i.e. age)

Figure S1

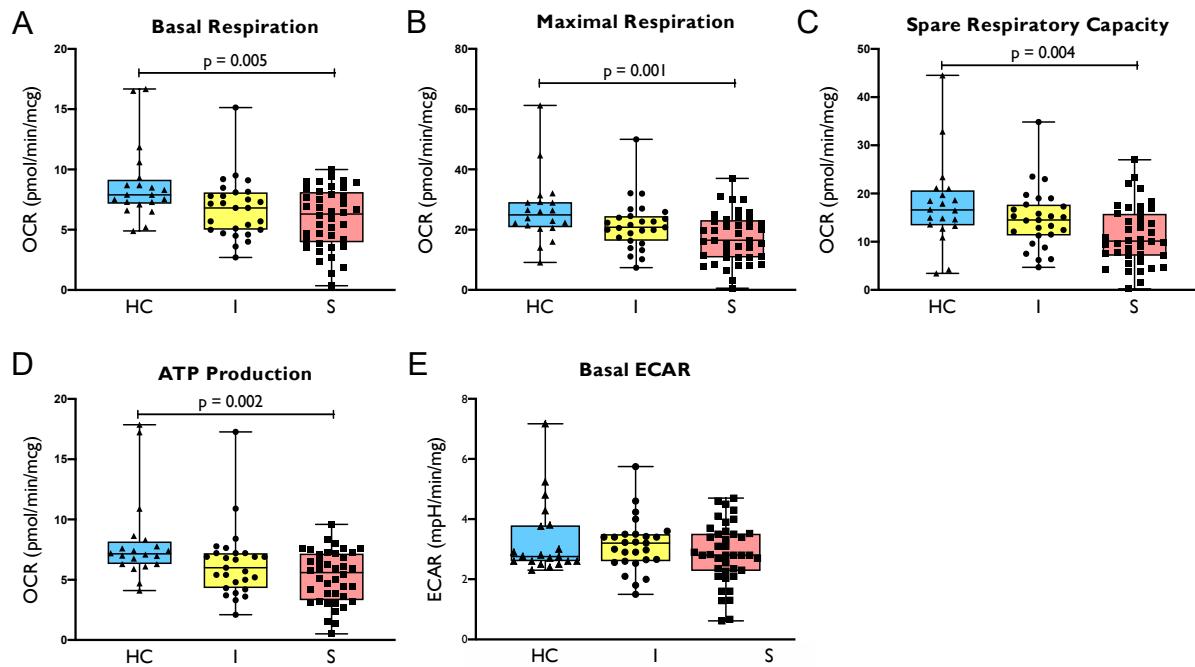


Figure S1 Oxygen consumption rate (OCR) (1A. basal respiration, 1B. maximal respiration, 1C. spare capacity, and 1D. ATP production) and extracellular acidification rate (ECAR) (1E) in healthy control/ HC ($n = 20$), uncomplicated infection/ I ($n = 27$) and sepsis/ S ($n = 40$) group. Comparison between groups were performed by one-way ANOVA followed by Tukey's multiple comparison test for maximal respiration and spare respiratory capacity; and Kruskal-Wallis test followed by Dunn's multiple comparison test for basal respiration, ATP production and ECAR.

Figure S2

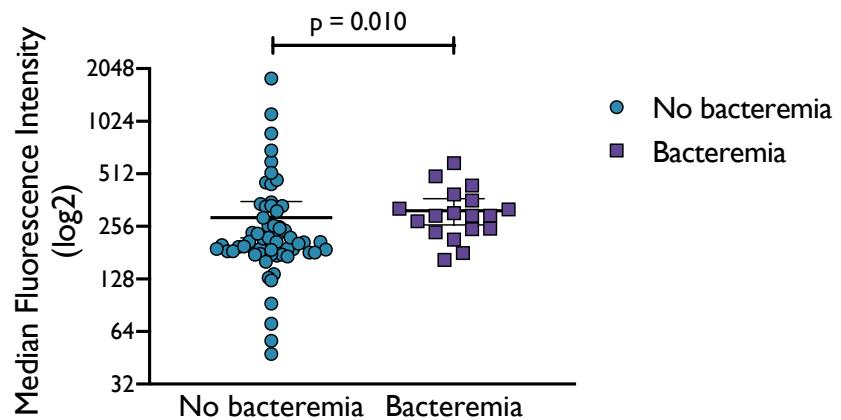


Figure S2 MitoSOX level in non-bacteremia (n = 62, including healthy controls) and bacteremia (n = 18) subjects. Comparison between groups was performed with Mann-Whitney U test.

Figure S3

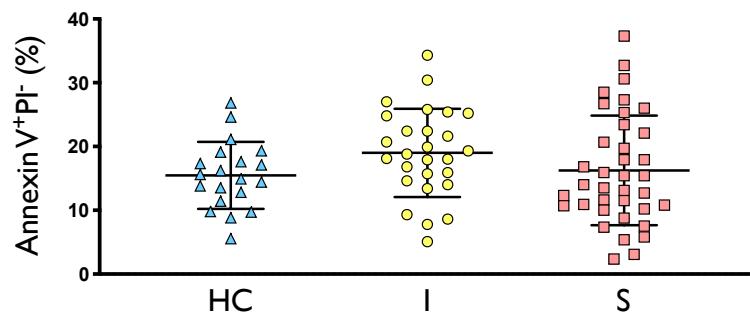


Figure S4 Annexin V⁺ and propidium iodide⁻ population in healthy control (HC), uncomplicated infection (I) and sepsis (S) (n = 20, 27, 37, respectively). Comparison between groups was made using one-way ANOVA followed by Tukey's multiple comparison.