

Discovery and validation of serum metabolic signature of neonatal sepsis.

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Fig. S1.

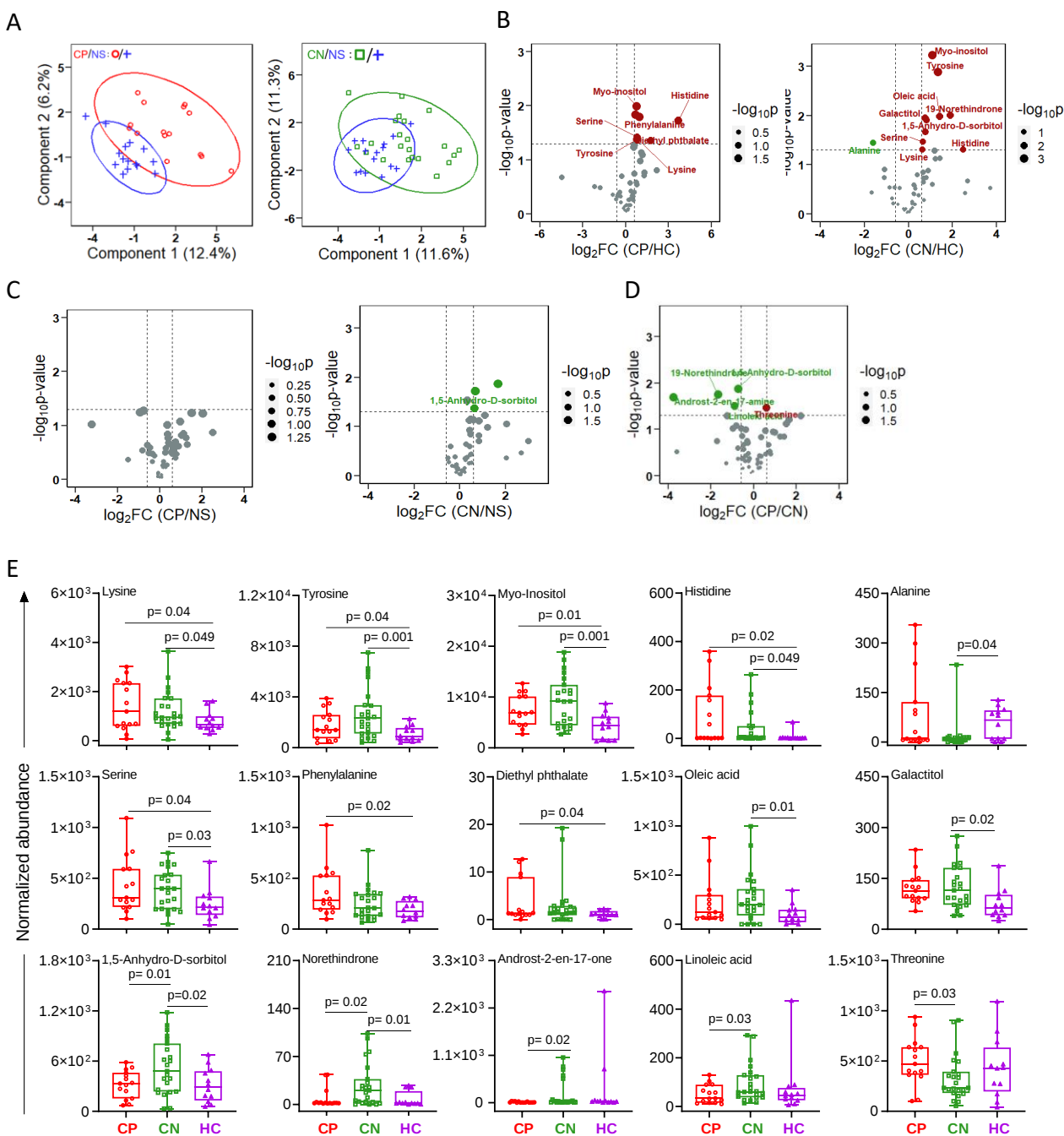


Figure S1. Serum metabolite profile of CP (n=16), CN (24), NS (n=17) neonatal sepsis cases and HC (n=14) in discovery set-I. (A) PLS-DA model depicting the group specific metabolic differences between CP, CN, and NS groups. (B-D) Volcano plots highlighting the deregulated metabolites that differentiate CP and CN from (B) HC, and (C) NS groups. (D) Volcano plot depicting the differential metabolites between CP and CN. (E) Box plots displaying the abundance of deregulated metabolites among CP, CN, and HC groups. PLS-DA: Partial Least Square-Discriminant Analysis, CP: culture-positive, CN: culture-negative, NS: no sepsis, HC: healthy control.

Fig. S2.

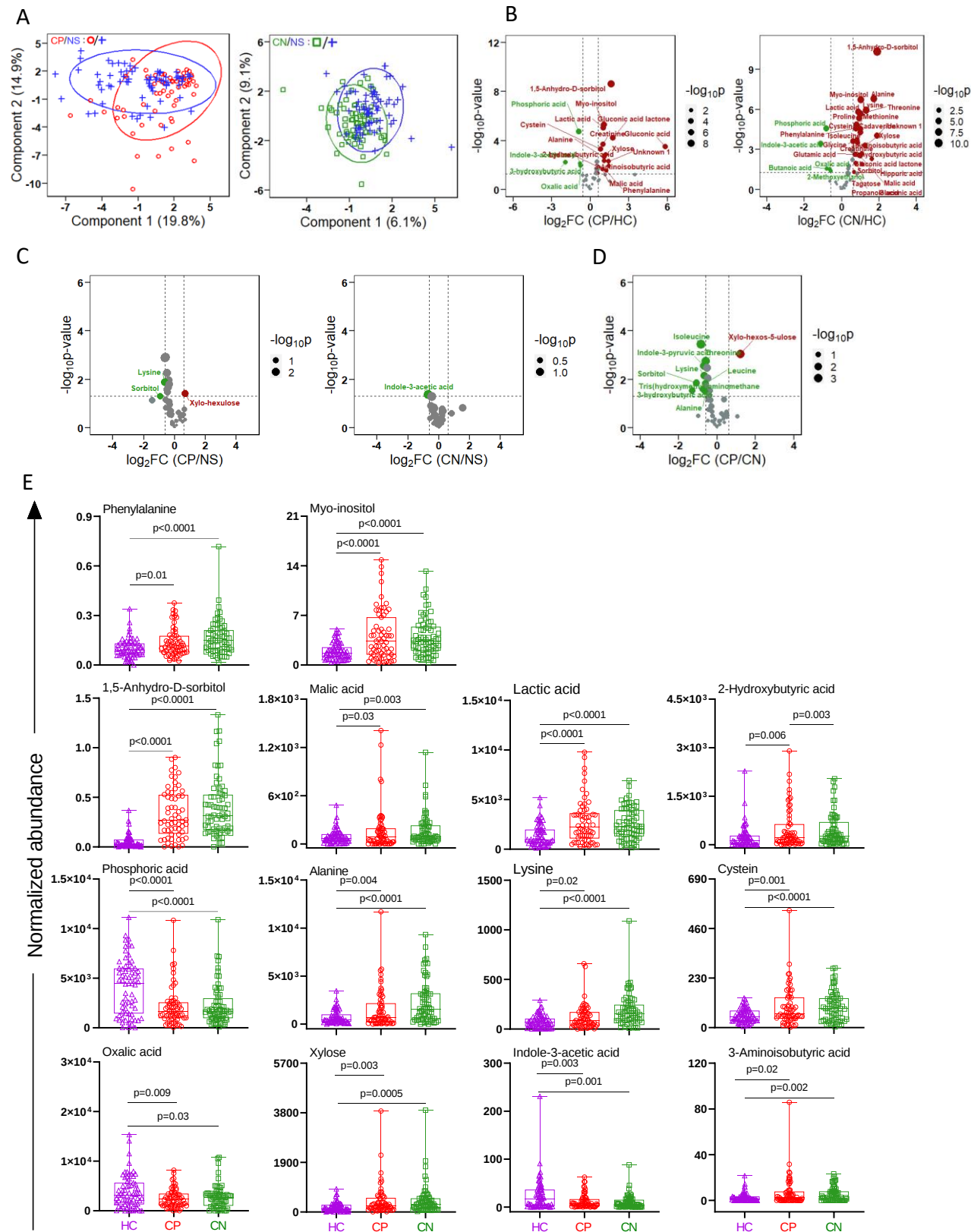
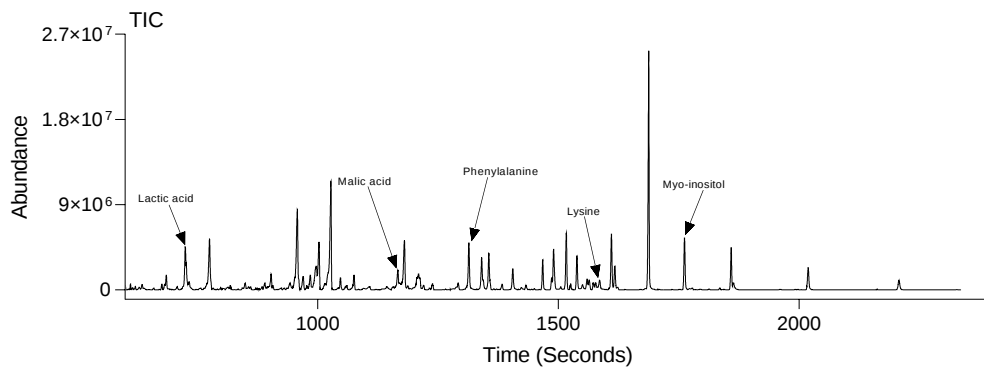


Figure S2. Serum metabolite profile of CP (n=63), CN (n=69), NS (n=68) sepsis cases and HC (n=69) in discovery set-II. (A) PLS-DA model depicting the group specific variation of CP and CN from NS group. (B-D) Volcano plots highlighting the deregulated metabolites that differentiate CP and CN from (B) HC (C) NS groups. (D) Volcano plot depicting the differential metabolites between CP and CN. (E) Box plots show displaying the abundance of deregulated metabolites among CP, CN, and HC groups. PLS-DA: Partial Least Square-Discriminant Analysis, CP: culture-positive, CN: culture-negative, NS: no sepsis, HC: healthy control.

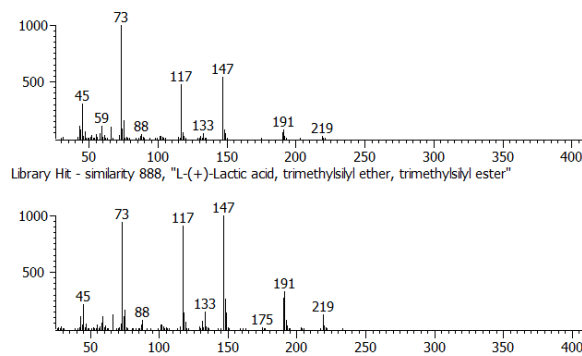
Fig. S3.

A



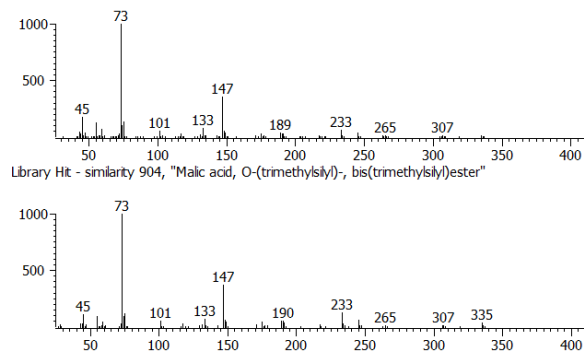
B

Peak True - sample "Sample", peak 55, at 724.4 s



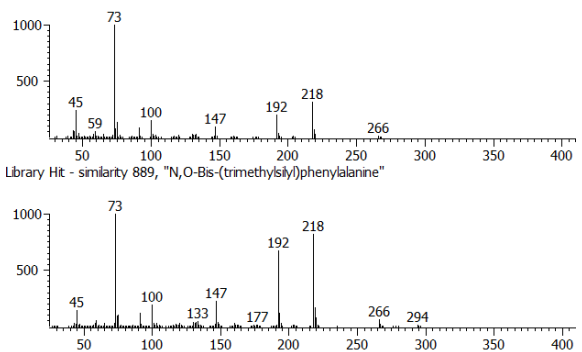
C

Peak True - sample "Sample", peak 306, at 1166.35 s



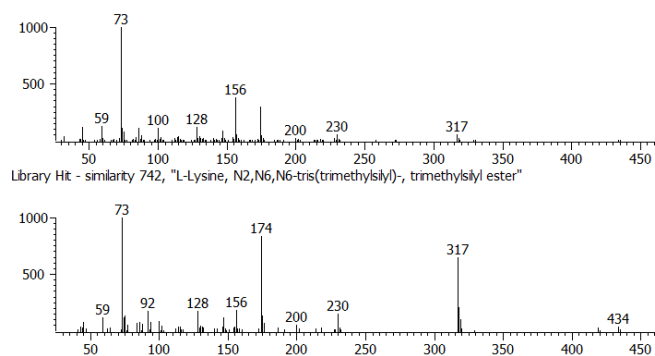
D

Peak True - sample "Sample", peak 433, at 1315.1 s



E

Peak True - sample "Sample", peak 572, at 1586.95 s



F

Peak True - sample "Sample", peak 622, at 1762.45 s

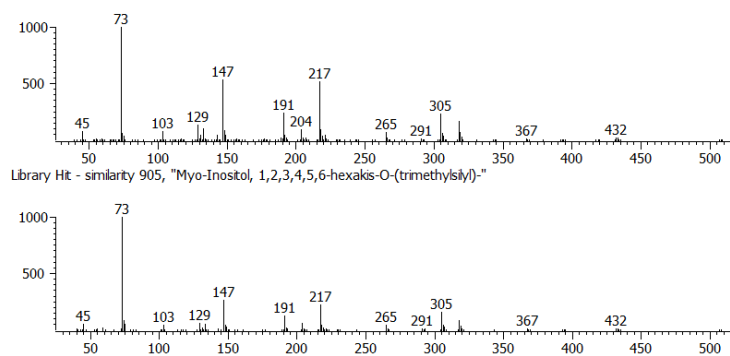


Figure S3. Confirmation of the identity of the key deregulated metabolites in neonatal sepsis subjects by running commercial standards. (A) Total ion chromatogram (TIC) of standard molecular mix reveals the identity of deregulated metabolites (B) Fragmentation pattern of lactic acid, (C) malic acid, (D) phenylalanine, (E) lysine, and (F) myo-inositol trimethylsilyl (TMS) derivatives.

Fig. S4.

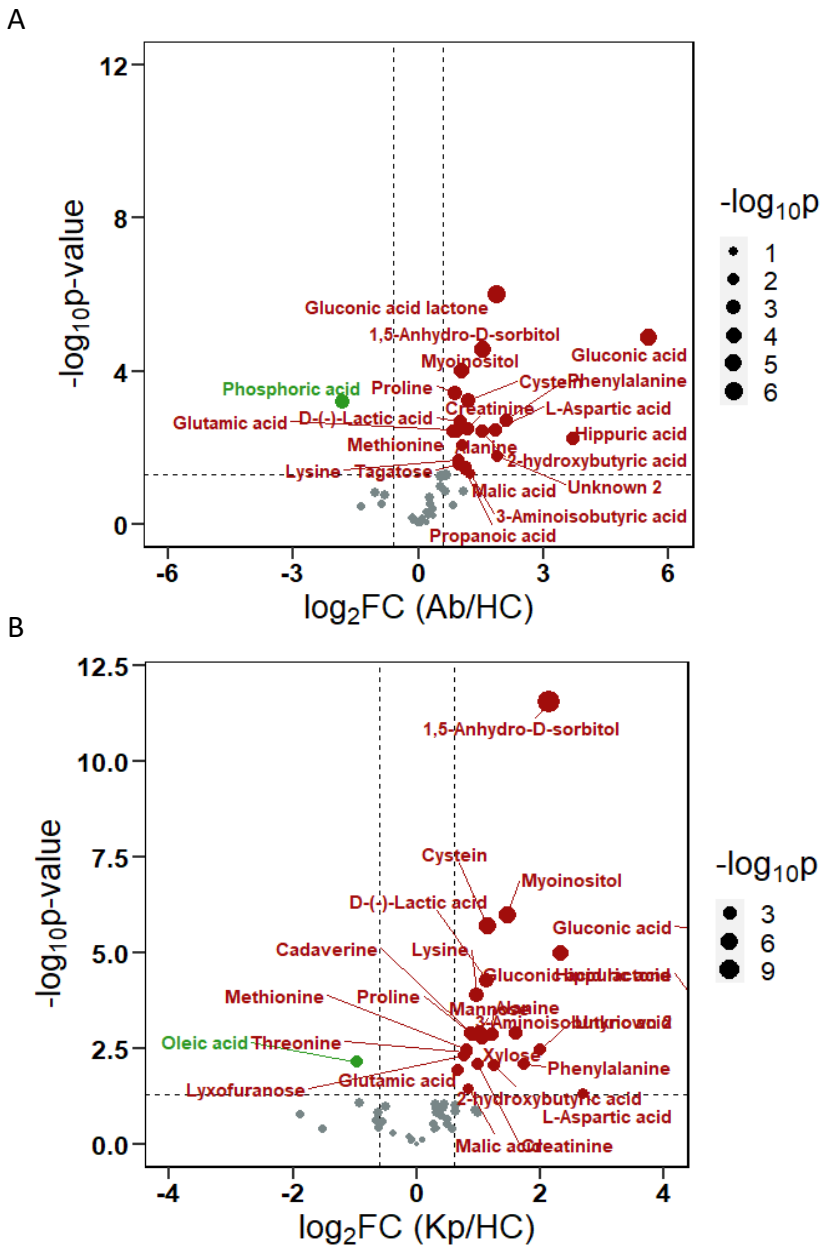


Figure S4. Metabolite profile of CP neonates infected with *Klebsiella pneumoniae* and *Acinetobacter baumannii* against HC. Volcano plots showing difference in the metabolite profile between (A) *Klebsiella pneumoniae* and HC, and (B) *Acinetobacter baumannii* and HC. CP: culture-positive, HC: healthy control.

Fig. S5.

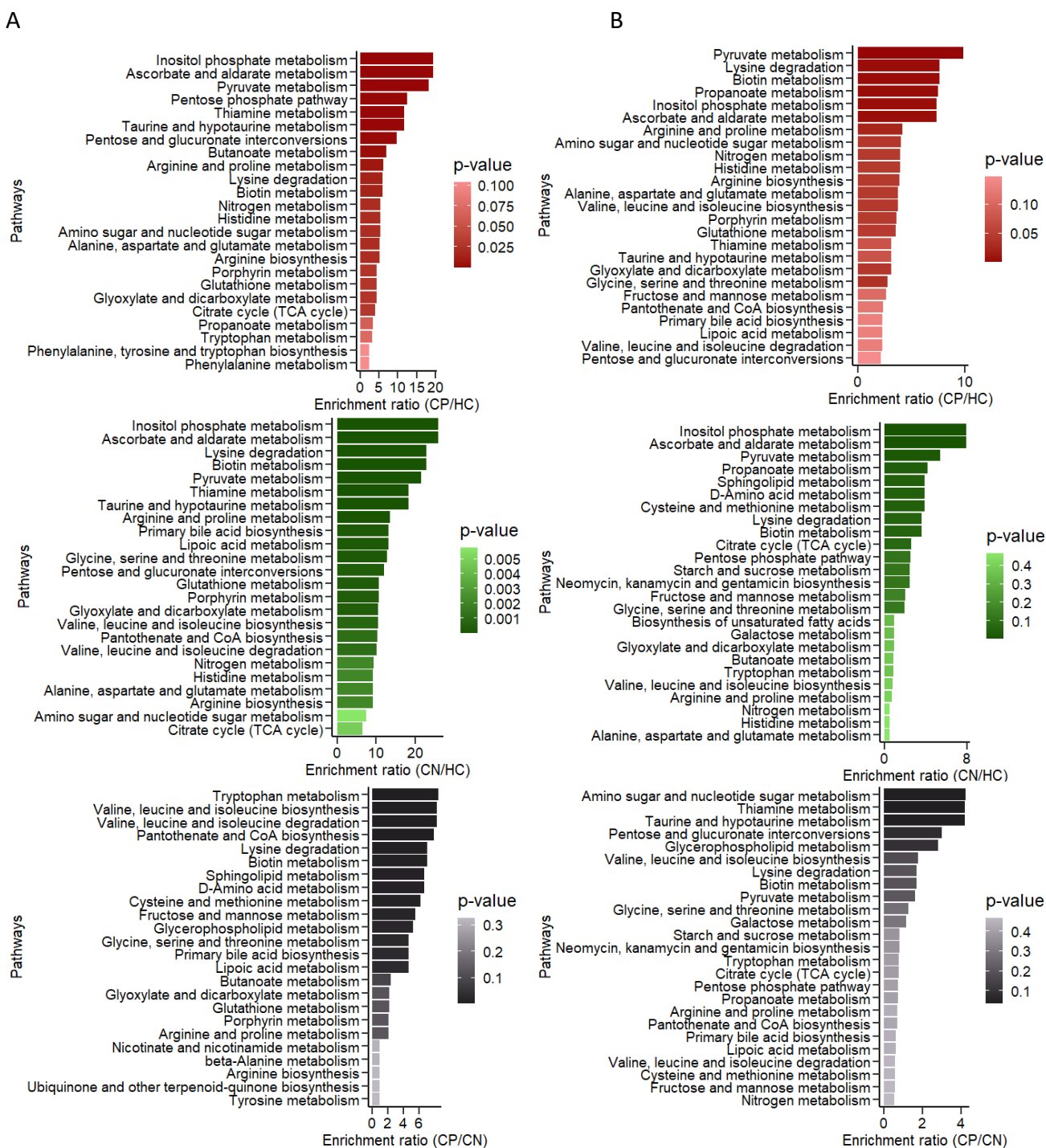


Figure S5. Metabolite Set Enrichment Analysis (MSEA) between sepsis and control groups. MSEA plot depicting the deregulated pathways between sepsis (CP, CN) and control groups (HC) in (A) Discovery set-I, and (B) Discovery set-II. CP: culture-positive, CN: culture-negative, HC: healthy controls.

Fig. S6.

A

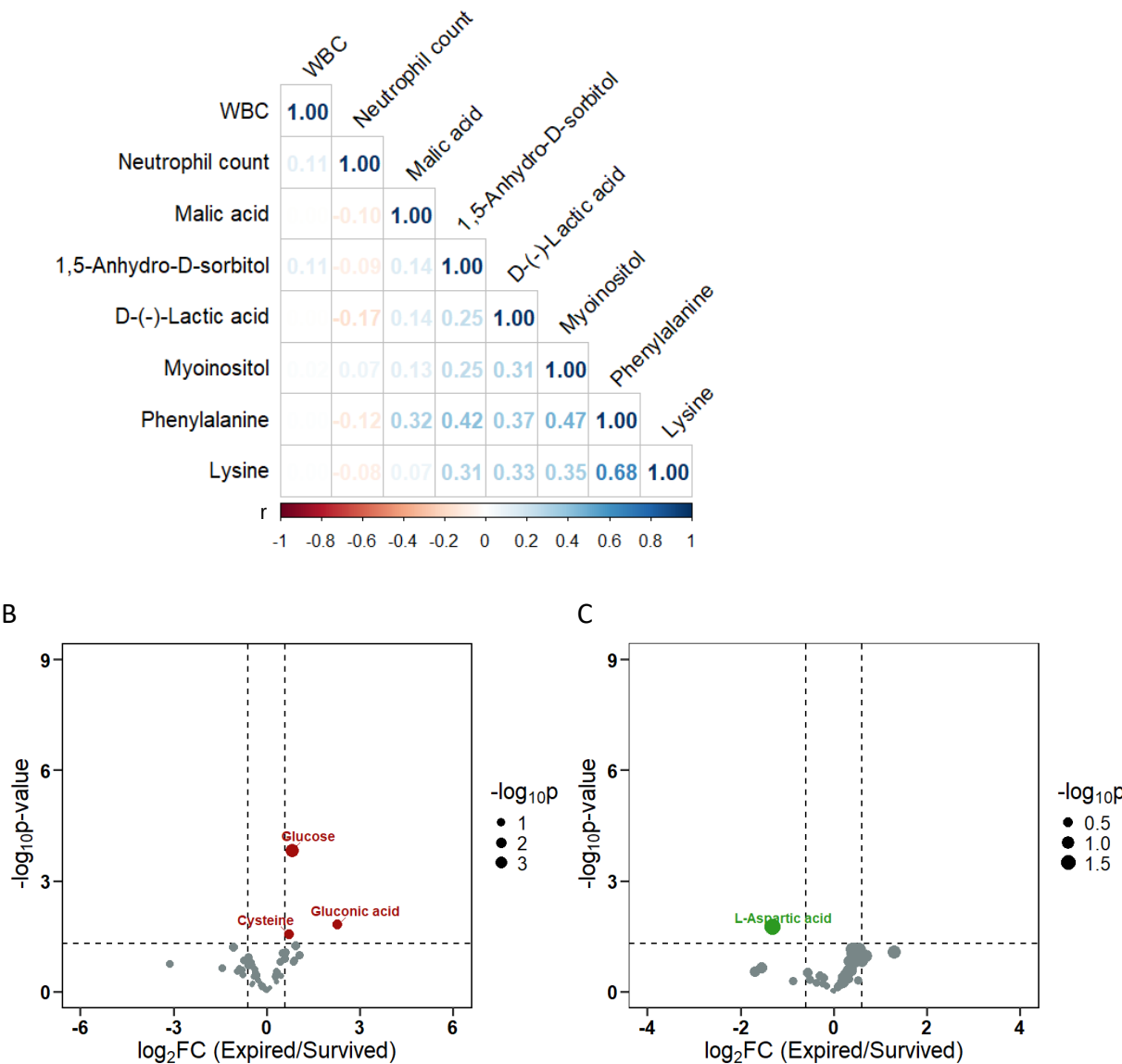


Figure S6. Correlation between sepsis serum metabolite signature and preexisting sepsis diagnostic parameters and putative serum metabolite markers for disease severity. (A) Correlation plot depicting the correlation between sepsis serum metabolite signature and WBC and neutrophil count which are quantified for sepsis diagnosis. **(B-C)** Volcano plot highlighting the metabolites that can differentiate neonates that expired from surviving neonates in **(B)** CP and **(C)** CN groups. r: Pearson's correlation coefficient, FC: Fold Change, WBC: White Blood Cells.

Fig. S7.

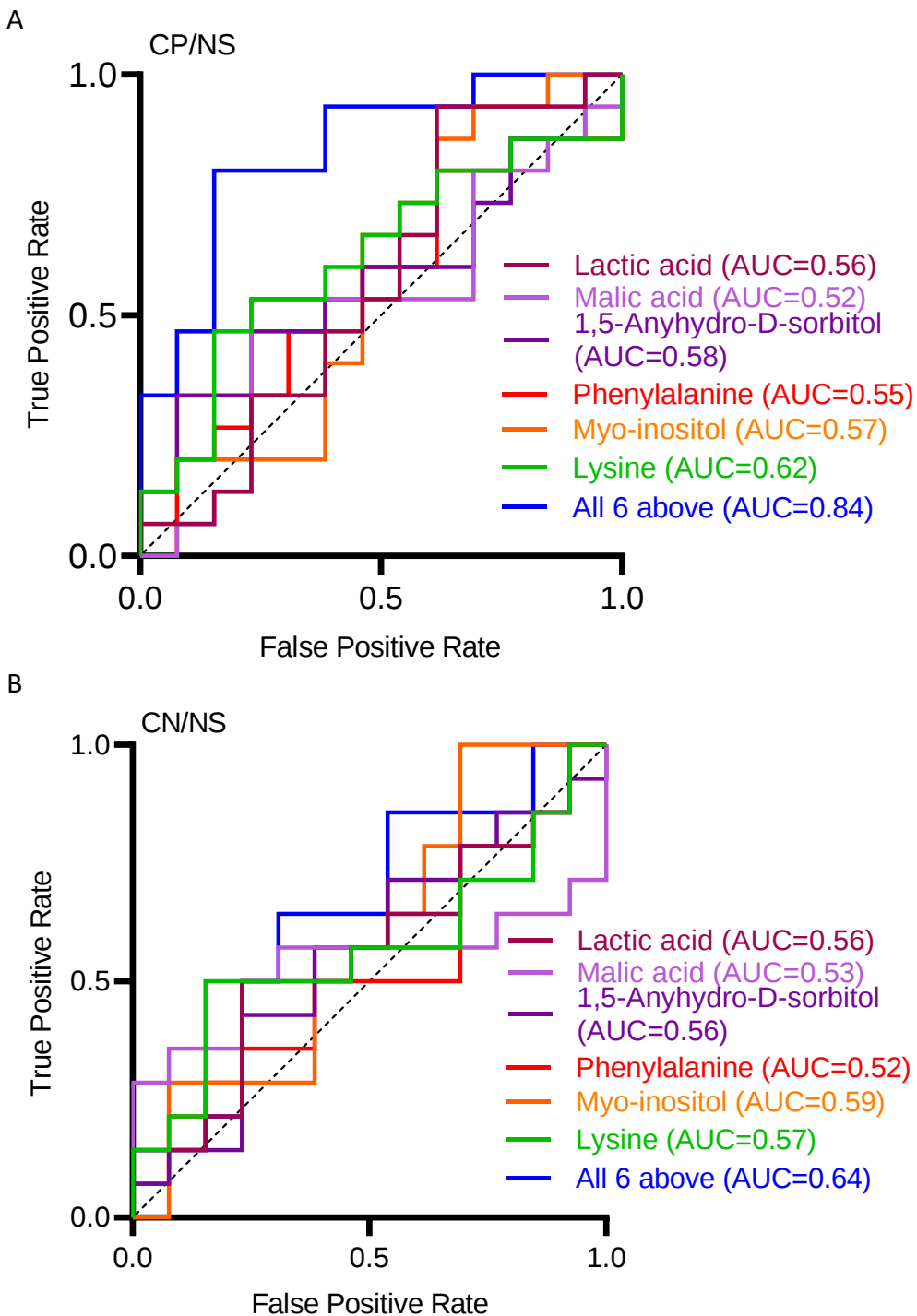


Figure S7. AUC of ROC of the biosignature consisting of 6 biomarkers depict high predictive ability for CP and CN cases against NS group (n=15 each). AUC of ROC in (A) CP against NS group, and (B) CN against NS group. AUC: area under the curve, ROC: receiver operating curve, CP: culture-positive, NS: no sepsis, CN: culture-negative.

Fig. S8.

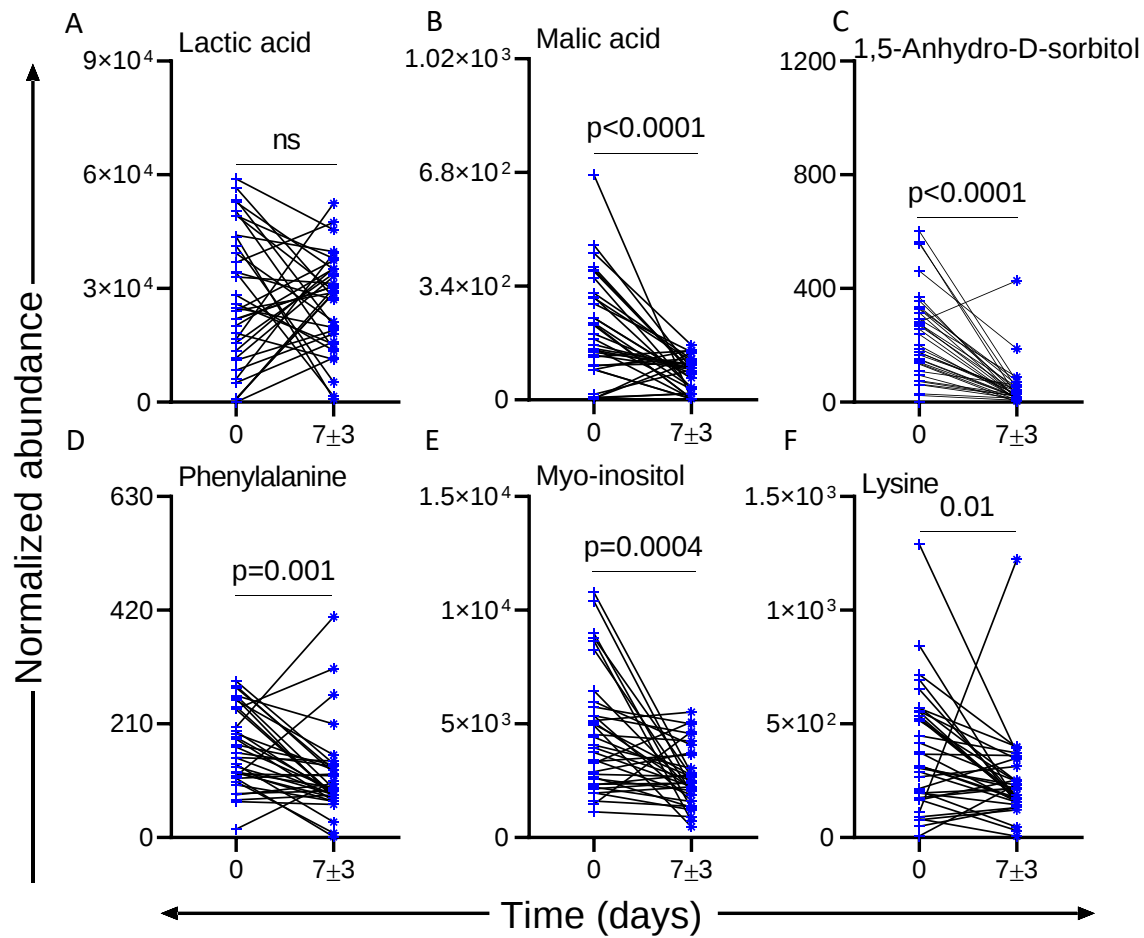


Figure S8. Trend in the abundance of deregulated metabolites in longitudinally followed up NS cases upon recovery. Dot plots depicting the trend of (A) Lactic acid, (B) Malic acid, (C) 1,5-Anhydro-D-sorbitol, (D) Phenylalanine, (E) Myo-inositol, and (F) Lysine in the NS group of an independent data set ($n=36$). NS, no sepsis.

Table S1. Group-specific epidemiological details and their significance in neonatal sepsis and control cases.

Clinical details	Total	Discovery set-I										Discovery set-II									
		Sub total	Sepsis		Control		p-value					Subtotal	Sepsis		Control		p-value				
			CP	CN	HC	NS	(CP/HC)	(CN/HC)	(CP/CN)	(CP/NS)	(CN/NS)		CP	CN	HC	NS	(CP/HC)	(CN/HC)	(CP/CN)	(CP/NS)	(CN/NS)
Number of neonates	500	71	16	24	14	17						269	63	69	69	68					
Gender (% female)	41.2, 206	42.3, 30	50, 8	41.7, 10	42.9, 6	35.3, 6	0.7	0.94	0.6	0.39	0.68	43.4, 117	31.7, 20	49.3, 34	50.7, 35	41.1, 28	0.02	0.86	0.04	0.23	0.38
Weight (mean±standard deviation in kg)	1.5 ±0.4	1.5 ±0.5	1.3 ±0.5	1.4 ±0.3	2 ±0.4	1.6 ±0.4	5E-04	1E-04	0.46	0.15	0.38	1.5 ±0.3	1.4 ±0.3	1.4 ±0.4	1.7 ±0.3	1.6 ±0.3	0.0001	3E-04	0.86	0.01	0.05
Mode of delivery (% Caesarean)	30.5, 40	32.4, 23	31.3, 5	23.8, 5	43.8, 7	33.3, 6	0.3	0.05	0.26	0.81	0.31	73.9, 155	31.7, 20	71.8, 28	43.5, 30	51.5, 35	0.16	0.86	0.22	0.02	0.26
Gestation (mean±standard deviation in weeks)	31.5 ±1.9	31.4 ±1.9	30.9 ±1.7	30.8 ±2.1	32.9 ±1.5	31.7 ±1.6	0.002	0.007	0.48	0.16	0.24	31.4 ±1.7	30.9 ±1.9	30.9 ±1.8	32.3 ±1.3	31.6 ±1.5	0.0001	1E-04	0.79	0.04	0.03
Outcome (% total)	23.7, 500	18.3, 71	43.8, 16	16.6, 24	-	11.8, 17	-	-	0.07	0.05	0.66	40.8, 250	77.3, 53	65, 63	1.5, 66	27.9, 68	0.0001	1E-04	0.15	0.001	1E-04
Difficulty breathing (% total)	65.2, 92	50, 52	43.8, 16	62.5, 22	-	33.3, 12	-	-	0.25	0.58	0.06	77.3, 181	81.1, 53	75.4, 61	-	76.1, 61	0.48	0.58	0.46	0.5	0.92
Apnea (% total)	18.8, 92	23.1, 52	18.8, 16	27.3, 22	50, 2	8.3, 12	-	-	0.54	0.45	0.22	6.5, 182	13.2, 53	3.2, 63	-	4.5, 66	0.37	0.12	0.06	0.1	0.68
WBC count (mean±standard deviation in 10 ⁹ /µL)	-	-	18.8 ±18	24.2 ±21.2	-	24.1 ±14.1	-	-	0.59	0.08	0.31	-	11.2 ±9.8	16.9 ±17.8	-	13.4 ±9.4	-	-	0.03	0.03	0.8
Absolute neutrophil count (mean±standard deviation in 10 ⁹)	-	-	3.8 ±2.5	3.7 ±2.5	-	6.9 ±3.4	-	-	0.97	0.03	0.03	-	4.8 ±3.1	4.2 ±3.8	-	4.1 ±2.0	-	-	0.34	0.71	0.25

Clinical details	Validation set										Follow-up set						
	Subtotal	Sepsis		Control		p-value					Subtotal	Sepsis		Control	p-value		
		CP	CN	HC	NS	(CP/HC)	(CN/HC)	(CP/CN)	(CP/NS)	(CN/NS)		CP	CN	NS	(CP/CN)	(CP/NS)	(CN/NS)
Number of neonates	60	15	15	15	15						100	29	35	36			
Gender (% female)	40, 24	60, 9	46.7, 7	20, 3	33.3, 5	0.7	0.94	0.6	0.27	0.46	46, 46	48.3, 14	40, 14	50, 18	0.51	0.89	0.4
Weight (mean±standard deviation in kg)	1.5 ±0.4	1.5 ±0.4	1.3 ±0.3	1.7 ±0.3	1.7 ±0.5	0.37	0.004	0.15	0.29	0.01	1.5 ±0.3	1.5 ±0.3	1.5 ±0.3	1.6 ±0.4	0.77	0.1	0.1
Mode of delivery (% Caesarean)	28.3, 17	40, 6	13.3, 2	26.7, 4	33.3, 5	0.44	0.37	0.11	0.71	0.21	46, 46	48.3, 14	51.4, 18	38.9, 14	0.8	0.45	0.29
Gestation (mean±standard deviation in weeks)	31.6 ±1.9	30.9 ±2	30.5 ±1.9	32.6 ±1.2	32.3 ±1.5	0.01	0.004	0.5	0.04	0.01	31.5 ±1.8	31.2 ±2	31.2 ±1.7	32 ±1.6	0.88	0.12	0.04
Outcome (% total)	30, 60	60, 15	60, 15	0	20, 15	-	-	1	0.03	0.03	-	-	-	-	-	-	-
Difficulty breathing (% total)	85, 40	100, 12	100, 13	-	33.3, 12	-	-	1	0.01	0.01	84, 99	86.2, 29	88.6, 35	80, 35	0.78	0.51	0.33
Apnea (% total)	12.5, 40	25, 12	7.7, 13	-	6.7, 15	-	-	0.63	0.21	0.92	11, 100	20.7, 29	11.4, 35	2.8, 35	0.32	0.05	0.19
WBC count (mean±standard deviation in 10 ⁹ /µL)	-	10.1 ±5	18.2 ±12.6	-	13.5 ±5.8	-	-	0.08	0.25	0.35	13.3 ±8.9	11.4 ±9	13.5 ±10.8	14.7 ±5.6	0.56	0.005	0.02
Absolute neutrophil count (Mean±standard deviation in 10 ⁹)	-	3.6 ±2.28	7.9 ±9.8	-	5.9 ±3.6	-	-	0.22	0.16	0.97	5.7 ±5.4	3.6 ±2.6	7.1 ±7.4	5.7 ±2.7	0.04	0.007	0.67

CP: Culture positive; CN: Culture negative; HC: Healthy control; NS: No sepsis

Table S3. Fold change and p-value of deregulated metabolites detected in serum samples that can differentiate sepsis from control groups.

Metabolic feature	Discovery set-I					Discovery set-II					Validation set				
	CP/HC	CN/HC	CP/CN	CP/NS	CN/NS	CP/HC	CN/HC	CP/CN	CP/NS	CN/NS	CP/HC	CN/HC	CP/CN	CP/NS	CN/NS
	log ₂ FC (p-value)														
1,5-Anhydro-D-sorbitol	0.04 (0.9)	0.8 (0.02)	-0.7 (0.01)	-0.08 (0.8)	0.6 (0.04)	1.6 (<0.0001)	1.9 (<0.0001)	-0.3 (0.1)	-0.3 (0.1)	0.1 (0.0004)	2.7 (0.0002)	2.3 (0.003)	0.4 (0.3)	0.3 (0.3)	-0.07 (0.9)
Lactic acid						1 (<0.0001)	0.9 (<0.0001)	0.1 (0.5)	0.1 (0.5)	1 (0.002)	1 (0.01)	1 (0.05)	-0.01 (1)	0.3 (0.5)	0.3 (0.6)
Lysine	0.8 (0.04)	0.6 (0.05)	0.2 (0.5)	0.4 (0.2)	0.2 (0.4)	0.6 (0.01)	1.3 (<0.0001)	-0.7 (0.01)	-0.6 (0.01)	0.02 (0.9)	1.6 (0.01)	1 (0.07)	0.6 (0.2)	0.6 (0.2)	-0.02 (1)
Malic acid						0.9 (0.02)	0.9 (0.003)	0.01 (1)	-0.05 (0.9)	-0.06 (0.8)	0.9 (0.0008)	1.4 (0.03)	-0.6 (0.2)	-0.06 (0.8)	0.5 (0.3)
Myo-inositol	0.8 (0.01)	1 (0.001)	-0.3 (0.2)	0.1 (0.6)	0.5 (0.07)	1 (<0.0001)	1 (<0.0001)	0.03 (0.9)	-0.03 (0.9)	-0.06 (0.7)	0.9 (0.01)	1 (0.01)	-0.08 (0.8)	-0.3 (0.3)	-0.2 (0.5)
Phenylalanine	1 (0.02)	0.4 (0.2)	0.6 (0.1)	0.4 (0.2)	-0.1 (0.6)	1.2 (0.03)	0.8 (<0.0001)	0.5 (0.3)	0.5 (0.3)	0.02 (0.9)	0.9 (0.01)	0.6 (0.1)	0.3 (0.3)	0.3 (0.4)	-0.08 (0.8)

Table S4. Criteria for suspicion and confirmation for neonatal sepsis cases and controls used in this study.

	Clinical criteria for suspicion of sepsis (any one or more)	Laboratory criteria for confirmed sepsis (any two parameters are considered for positive screening)
1	Difficulty feeding	White blood cell (WBC) count (absolute numbers/ microlitre) $<4.0 \times 10^9$ cells/L
2	Convulsions or abnormal posturing	Absolute neutrophil count $< 1.5 \times 10^9$ cells/L
3	No movement or movement only when stimulated OR significant lethargy	C-reactive protein (CRP) absolute value in mg/L > 6 mg/L
4	Fever	Total leucocyte count (TLC) < 5000 /cmm
5	Cold to touch	Band to total polymorph ratio >2
6	New onset or increased apnea	Micro ESR > 15 mm in 1 st hour
7	Bleeding from the gastrointestinal tract	
8	Hypotonia or floppiness	
9	Difficulty breathing	
10	Bloody/bilious vomiting	
11	Diarrhea (watery stool)	
12	Pus from the umbilical stump	
13	Discharge from ear	
14	Mottling	
15	Bleeding from skin/mucosa/lungs	
16	Others (Specify)	
17	Temperature $>37.5^\circ\text{C}$	
18	Heart Rate > 180 /min	
19	Capillary refill time (CRT) > 3 sec	
20	Respiratory rate > 60 /min	
21	Severe chest in-drawing	
22	Movement only when stimulated	
23	Cyanosis	
24	Sclerema	
25	Temperature $<36.5^\circ\text{C}$	
26	Heart Rate < 100 /min	
27	Grunting	
28	Bulging fontanelle	
29	Abdominal distension	
30	Abdominal erythema	
31	Abdominal tenderness	
32	Meningitis	
33	PNEUMONIA	
34	Peritonitis	
35	Necrotizing enterocolitis	

Table S5. Definition used for classification of the neonatal sepsis suspected cases.

Diagnosis	Working definitions
Culture positive sepsis (CP)	Baby unwell/maternal-perinatal risk factors AND must meet ANY ONE of the following criteria 1. True pathogen detected: Baby has a recognized pathogen cultured from 1 or more blood cultures and organism cultured is not related to an infection at another site 2. CoNS: CoNS is cultured from 1 or more blood samples drawn on separate occasions and organism cultured is not related to an infection at another site AND Physician institutes appropriate treatment OR death/LAMA/referred before completing appropriate therapy.
Culture negative sepsis (CN)	Baby unwell/maternal-perinatal risk factors AND all of the following criteria: 1. Blood culture not done or no organisms detected in blood. 2. Positive septic screen OR physician institutes appropriate treatment for sepsis OR death/LAMA/referred before completing appropriate treatment.
No sepsis (NS)	Baby unwell/maternal-perinatal risk factors AND following criteria: 1. Negative septic screen and blood culture 2. Physician did not treat/stopped treatment before 5 days as it was not considered to be sepsis