

Composition of early life leukocyte populations in preterm infants with and without late-onset sepsis

Supporting Information

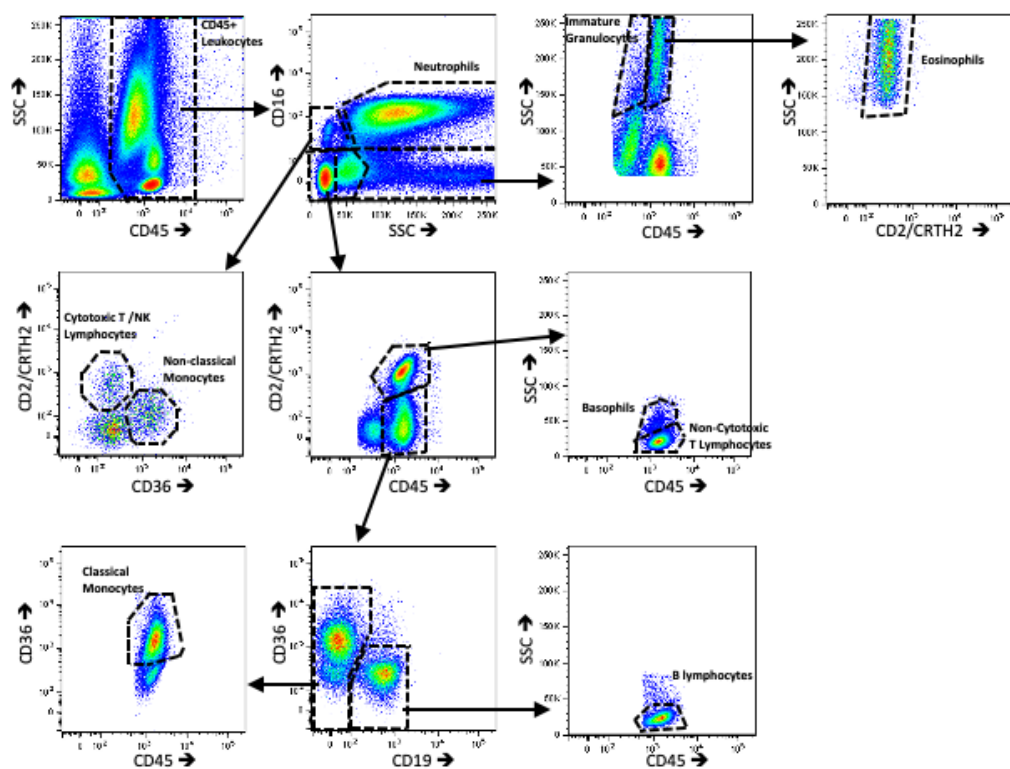


Figure S1. Leukocyte population gating strategy. Example plots of CD45⁺ leukocytes, neutrophils, basophils, immature granulocytes, eosinophils, classical and non-classical monocytes, non-cytotoxic and cytotoxic T/NK lymphocytes, and B lymphocytes from preterm infant peripheral blood.

Supplementary Table 1. Descriptive summaries of absolute counts and percentage of total CD45⁺ leukocyte populations in preterm infants with and without LOS. Median (95% CI) absolute counts (x 10⁹/L) are presented in the first row and frequencies (%) in the second row of each timepoint.

	Any LOS n=43	Confirmed LOS n=28	Clinical LOS n=15	No LOS n=76
CD45⁺ Leukocytes				
Day 1	5.41 (4.03, 7.95)	5.30 (3.65, 7.46)	7.32 (3.84, 11.8)	5.83 (4.81, 6.68)
Day 7	6.79 (4.51, 9.08)	5.30 (4.11, 7.49)	9.08 (6.95, 11.2)	6.11 (5.19, 6.89)
Day 14	6.00 (5.05, 7.24)	5.46 (4.68, 8.24)	6.41 (5.57, 7.54)	6.29 (5.59, 7.32)
Day 21	5.73 (4.86, 6.68)	5.68 (4.30, 6.81)	6.14 (4.84, 7.86)	5.45 (4.97, 6.27)
Day 28	5.89 (4.35, 6.95)	4.70 (3.65, 6.35)	7.54 (3.68, 8.51)	5.76 (5.54, 6.49)
Neutrophils				
Day 1	2.15 (1.19, 3.38)	2.17 (1.17, 4.14)	2.11 (0.90, 5.05)	2.17 (1.72, 2.78)
Day 7	40.4 (36.1, 47.4)	41.2 (34.7, 48.0)	39.8 (17.9, 53.0)	38.4 (35.4, 43.3)
Day 14	1.96 (1.32, 4.38)	1.51 (0.83, 3.49)	4.38 (2.58, 6.68)	2.08 (1.55, 2.41)
Day 21	35.0 (28.6, 46.6)	28.9 (23.6, 39.4)	48.1 (31.4, 65.3)	32.8 (27.1, 35.8)
Day 28	2.48 (1.96, 3.70)	2.21 (1.31, 3.78)	2.89 (2.12, 3.73)	2.42 (2.03, 2.92)
Day 1	39.3 (35.4, 50.5)	37.5 (31.1, 50.5)	39.4 (38.1, 52.3)	38.4 (34.2, 42.6)
Day 7	2.09 (1.63, 2.46)	2.12 (1.49, 2.92)	2.02 (1.60, 2.68)	1.49 (1.25, 1.79)
Day 14	36.4 (32.7, 40.2)	37.5 (31.4, 43.9)	34.2 (31.3, 40.5)	29.5 (27.4, 32.0)
Day 21	1.77 (1.37, 2.69)	1.57 (1.07, 2.22)	3.30 (0.66, 5.38)	1.29 (1.12, 1.66)
Day 28	36.5 (28.7, 41.0)	33.3 (26.31, 40.1)	43.6 (23.0, 63.2)	23.2 (20.6, 27.1)
Basophils				
Day 1	0.03 (0.02, 0.04)	0.03 (0.02, 0.04)	0.02 (0.02, 0.04)	0.02 (0.02, 0.03)
Day 7	0.40 (0.30, 0.52)	0.38 (0.33, 0.59)	0.38 (0.20, 0.52)	0.40 (0.41, 0.52)
Day 14	0.03 (0.03, 0.05)	0.03 (0.03, 0.06)	0.04 (0.02, 0.07)	0.04 (0.04, 0.05)
Day 21	0.55 (0.43, 0.78)	0.64 (0.43, 0.91)	0.49 (0.30, 0.79)	0.70 (0.62, 0.83)
Day 28	0.03 (0.02, 0.04)	0.03 (0.02, 0.05)	0.03 (0.02, 0.04)	0.05 (0.04, 0.06)
Day 1	0.60 (0.39, 0.73)	0.59 (0.44, 0.80)	0.39 (0.24, 0.90)	0.70 (0.60, 0.87)
Day 7	0.03 (0.02, 0.04)	0.03 (0.02, 0.04)	0.03 (0.02, 0.05)	0.03 (0.03, 0.04)
Day 14	0.50 (0.39, 0.63)	0.48 (0.37, 0.71)	0.41 (0.25, 0.70)	0.65 (0.56, 0.70)
Day 21	0.02 (0.02, 0.04)	0.02 (0.02, 0.04)	0.03 (0.02, 0.04)	0.03 (0.03, 0.03)
Day 28	0.40 (0.36, 0.59)	0.48 (0.31, 0.73)	0.42 (0.23, 0.94)	0.60 (0.54, 0.66)
Immature granulocytes				
Day 1	0.10 (0.06, 0.27)	0.09 (0.05, 0.20)	0.21 (0.06, 1.59)	0.13 (0.09, 0.20)
Day 7	2.40 (1.37, 2.99)	1.81 (1.21, 2.93)	2.69 (1.97, 14.0)	2.30 (1.66, 3.15)
Day 14	0.19 (0.11, 0.30)	0.17 (0.09, 0.30)	0.22 (0.12, 0.61)	0.14 (0.10, 0.21)
Day 21	3.05 (1.91, 4.86)	2.95 (1.75, 5.75)	4.67 (1.77, 6.58)	2.20 (1.84, 2.83)
Day 28	0.18 (0.06, 0.15)	0.17 (0.09, 0.27)	0.22 (0.05, 0.28)	0.08 (0.06, 0.11)
Day 1	3.00 (1.71, 4.30)	2.68 (1.71, 5.25)	3.10 (0.80, 4.30)	1.60 (1.02, 1.96)
Day 7	0.11 (0.03, 0.13)	0.10 (0.04, 0.21)	0.11 (0.06, 0.27)	0.05 (0.04, 0.07)
Day 14	1.77 (1.09, 2.64)	1.64 (0.65, 3.52)	2.09 (0.81, 3.27)	1.10 (0.74, 1.35)
Day 21	0.06 (0.09, 0.20)	0.04 (0.03, 0.13)	0.09 (0.03, 0.30)	0.05 (0.04, 0.06)
Day 28	1.10 (0.77, 1.84)	0.90 (0.55, 1.51)	1.84 (1.05, 3.22)	0.90 (0.62, 1.28)
Eosinophils				
Day 1	0.02 (0.01, 0.03)	0.01 (0.00, 0.02)	0.03 (0.02, 0.07)	0.03 (0.02, 0.03)
Day 7	0.20 (0.13, 0.62)	0.14 (0.90, 0.41)	0.62 (0.19, 1.03)	0.50 (0.31, 0.69)
Day 14	0.11 (0.07, 0.15)	0.10 (0.06, 0.15)	0.15 (0.06, 0.24)	0.12 (0.09, 0.17)
Day 21	1.85 (1.00, 2.61)	1.80 (0.94, 2.61)	2.48 (0.74, 4.79)	2.40 (1.65, 2.98)
Day 28	0.16 (0.05, 0.23)	0.14 (0.04, 0.21)	0.18 (0.05, 0.40)	0.10 (0.07, 0.12)
Day 1	2.30 (1.08, 3.54)	2.52 (0.55, 3.54)	2.35 (1.76, 6.33)	1.50 (1.24, 1.96)
Day 7	0.20 (0.15, 0.32)	0.20 (0.12, 0.31)	0.20 (0.13, 0.58)	0.17 (0.13, 0.21)
Day 14	3.20 (2.47, 3.65)	3.17 (2.27, 5.80)	3.26 (2.34, 8.07)	3.00 (2.42, 4.10)

Day 28	0.16 (0.13, 0.24) 3.08 (2.35, 3.96)	0.16 (0.11, 0.31) 3.09 (2.33, 4.94)	0.18 (0.06, 0.33) 2.80 (1.94, 7.29)	0.16 (0.14, 0.23) 3.10 (2.30, 4.09)
Classical monocytes				
Day 1	0.40 (0.22, 0.61) 6.61 (5.54, 7.56)	0.28 (0.22, 0.61) 6.28 (4.64, 7.51)	0.43 (0.14, 1.13) 6.94 (3.12, 9.83)	0.45 (0.38, 0.53) 7.45 (6.22, 8.60)
Day 7	0.61 (0.48, 0.78) 8.75 (6.36, 11.3)	0.61 (0.36, 0.84) 8.95 (5.85, 11.7)	0.61 (0.38, 0.78) 8.52 (3.77, 14.0)	0.59 (0.49, 0.68) 8.30 (6.57, 10.9)
Day 14	0.42 (0.34, 0.61) 7.60 (5.68, 8.38)	0.39 (0.27, 0.54) 6.80 (4.98, 8.33)	0.60 (0.32, 0.69) 8.14 (5.86, 10.2)	0.61 (0.49, 0.71) 8.55 (7.35, 9.48)
Day 21	0.44 (0.32, 0.58) 6.90 (6.01, 8.20)	0.42 (0.23, 0.58) 6.60 (5.61, 7.61)	0.45 (0.32, 0.69) 8.42 (5.54, 11.3)	0.39 (0.32, 0.50) 7.40 (6.40, 8.31)
Day 28	0.45 (0.35, 0.53) 7.95 (6.91, 8.55)	0.41 (0.26, 0.54) 7.28 (6.31, 11.1)	0.47 (0.35, 0.79) 8.07 (5.37, 9.15)	0.46 (0.38, 0.54) 7.80 (6.74, 9.02)
Non-classical monocytes				
Day 1	0.05 (0.04, 0.09) 0.90 (0.69, 1.17)	0.05 (0.03, 0.09) 0.79 (0.60, 1.12)	0.05 (0.03, 0.15) 1.08 (0.67, 1.31)	0.07 (0.05, 0.09) 0.95 (0.84, 1.27)
Day 7	0.06 (0.05, 0.10) 0.90 (0.60, 1.33)	0.06 (0.03, 0.10) 0.93 (0.36, 1.62)	0.06 (0.05, 0.12) 0.90 (0.60, 1.82)	0.06 (0.05, 0.08) 1.00 (0.79, 1.20)
Day 14	0.08 (0.05, 0.12) 1.24 (0.96, 1.72)	0.06 (0.04, 0.10) 1.05 (0.77, 1.38)	0.12 (0.06, 0.15) 1.72 (0.96, 3.01)	0.09 (0.07, 0.12) 1.40 (1.19, 1.60)
Day 21	0.08 (0.06, 0.13) 1.40 (1.14, 1.84)	0.08 (0.05, 0.13) 1.37 (1.05, 1.84)	0.12 (0.06, 0.18) 1.68 (1.00, 2.79)	0.06 (0.05, 0.09) 1.10 (0.94, 1.42)
Day 28	0.08 (0.06, 0.11) 1.64 (1.31, 1.98)	0.07 (0.06, 0.11) 1.68 (1.13, 2.10)	0.11 (0.04, 0.20) 1.54 (1.10, 2.29)	0.07 (0.06, 0.10) 1.50 (1.21, 1.70)
Non-cytotoxic T lymphocytes				
Day 1	0.92 (0.61, 1.09) 13.6 (11.7, 17.9)	0.89 (0.41, 1.11) 14.9 (11.7, 22.1)	1.02 (0.53, 1.36) 13.1 (7.48, 21.4)	0.95 (0.83, 1.26) 19.5 (16.9, 21.1)
Day 7	1.10 (0.87, 1.32) 20.3 (13.8, 24.9)	1.27 (0.87, 1.86) 24.9 (17.1, 32.4)	0.96 (0.66, 1.31) 12.0 (8.13, 20.9)	1.51 (1.27, 2.00) 27.0 (24.4, 29.9)
Day 14	1.07 (0.83, 1.38) 15.5 (13.5, 24.7)	1.21 (0.81, 1.60) 16.7 (13.0, 26.3)	0.86 (0.66, 1.31) 14.5 (12.0, 21.7)	1.55 (1.23, 1.76) 25.2 (20.2, 27.3)
Day 21	1.11 (0.91, 1.67) 18.6 (14.7, 25.4)	1.62 (0.94, 1.79) 22.9 (15.1, 27.5)	1.01 (0.46, 1.17) 15.2 (9.39, 20.8)	1.71 (1.43, 1.95) 30.1 (26.5, 33.0)
Day 28	1.15 (0.76, 1.42) 21.7 (16.6, 29.1)	1.27 (0.75, 1.60) 23.5 (16.6, 30.1)	0.93 (0.44, 1.85) 19.2 (9.22, 31.9)	1.95 (1.63, 2.15) 32.4 (30.3, 35.8)
Cytotoxic T/NK lymphocytes				
Day 1	0.03 (0.02, 0.05) 0.50 (0.28, 0.77)	0.03 (0.02, 0.04) 0.53 (0.27, 0.77)	0.05 (0.03, 0.10) 0.64 (0.25, 1.10)	0.03 (0.03, 0.05) 0.50 (0.46, 0.77)
Day 7	0.02 (0.02, 0.03) 0.40 (0.31, 0.48)	0.02 (0.01, 0.03) 0.40 (0.23, 0.55)	0.02 (0.01, 0.06) 0.34 (0.18, 0.65)	0.03 (0.03, 0.04) 0.50 (0.39, 0.57)
Day 14	0.04 (0.02, 0.05) 0.50 (0.33, 0.75)	0.03 (0.02, 0.05) 0.44 (0.27, 0.71)	0.04 (0.01, 0.07) 0.74 (0.20, 1.04)	0.03 (0.03, 0.04) 0.50 (0.47, 0.59)
Day 21	0.05 (0.04, 0.06) 0.90 (0.69, 1.18)	0.06 (0.04, 0.09) 0.99 (0.70, 1.38)	0.04 (0.02, 0.06) 0.60 (0.30, 1.13)	0.04 (0.03, 0.05) 0.75 (0.57, 0.87)
Day 28	0.05 (0.03, 0.06) 1.00 (0.74, 1.27)	0.06 (0.04, 0.09) 1.12 (0.74, 1.41)	0.05 (0.03, 0.07) 0.75 (0.39, 1.32)	0.06 (0.05, 0.08) 1.10 (0.80, 1.22)
B lymphocytes				
Day 1	0.20 (0.16, 0.30) 3.10 (2.50, 4.95)	0.19 (0.16, 0.35) 3.66 (2.11, 6.79)	0.25 (0.11, 0.33) 2.73 (2.16, 5.01)	0.21 (0.18, 0.26) 3.85 (2.97, 4.56)
Day 7	0.22 (0.13, 0.32) 2.90 (2.48, 3.73)	0.23 (0.13, 0.38) 3.18 (2.64, 5.29)	0.17 (0.08, 0.35) 2.44 (1.07, 3.68)	0.25 (0.19, 0.30) 3.60 (3.10, 4.08)
Day 14	0.23 (0.18, 0.31) 4.20 (3.18, 5.52)	0.26 (0.19, 0.40) 4.47 (3.43, 5.79)	0.19 (0.13, 0.33) 2.77 (2.02, 6.98)	0.30 (0.22, 0.35) 4.25 (3.04, 5.60)
Day 21	0.28 (0.18, 0.38) 4.60 (3.20, 5.52)	0.31 (0.18, 0.50) 5.01 (3.27, 7.41)	0.21 (0.12, 0.38) 3.35 (2.46, 4.73)	0.25 (0.20, 0.34) 4.10 (3.47, 5.33)

Day 28	0.25 (0.21, 0.35)	0.31 (0.13, 0.49)	0.23 (0.13, 0.28)	0.37 (0.23, 0.45)
	4.45 (3.21, 5.96)	5.65 (3.31, 7.93)	3.21 (2.33, 5.93)	6.40 (4.62, 7.59)

Supplementary Table 2. Basic clinical characteristics of the infants with confirmed and clinical LOS

	Confirmed LOS n=28	Clinical LOS n=15	P value
Gestational age (weeks)	26.6 (25.4-28.3)	25.4 (24.7-26.4)	0.069
Birthweight (grams)	835 (690-1028)	705 (600-865)	0.119
Birthweight z-score	-0.06 (-0.75-0.21)	-0.04 (-0.98-0.65)	0.855
Males	15 (53.6)	7 (46.7)	0.755
Multiple birth	7 (25.0)	2 (13.3)	0.458
Caesarean section	16 (57.1)	9 (60.0)	>0.999
ROM	8 (28.6)	9 (60.0)	0.057
Antenatal steroids	25 (89.3)	15 (100)	0.302
Apgar <7 at 5 min	8 (28.6)	4 (26.7)	>0.999
Chorioamnionitis [^]	9/26 (34.6)	9/14 (64.3)	0.101
Mechanical ventilation	27 (96.4)	14 (93.3)	>0.999
Duration (hours)	177 (27-773)	393 (153-1337)	0.116
Continuous positive airway pressure	28 (100)	15 (100)	>0.999
Duration (hours)	845 (642-1243)	952 (655-1164)	0.990
IVH (grade III/IV)	1 (3.6)	3 (20.0)	0.114
Chronic lung disease	8 (28.6)	7 (46.7)	0.318
ROP (stage III/IV) [^]	1/25 (4.0)	1/15 (6.7)	>0.999
Length of NICU stay (days)	99 (60-117)	105 (94-164)	0.182
Mortality to 28 days	0 (0)	0 (0)	>0.999
Age at sepsis onset (days)	11 (9-16)	16 (10-20)	0.130
Highest CRP (mg/L)*	57(31-91)	26(19-45)	0.004

Data are expressed as median (IQR) or n (%), as appropriate. [^]from available reports. * Within 72 hour of blood culture. ROM, rupture of membranes >24 hours prior to delivery; IVH, intraventricular haemorrhage; ROP, retinopathy of prematurity. CRP, C-reactive protein.

Supplementary Table 3. Descriptive summaries of adjusted absolute counts and percentage of total CD45⁺ leukocyte populations in preterm infants with and without LOS. Estimated marginal mean (95% CI) absolute counts (x 10⁹/L) are presented in the first row and frequencies (%) in the second row of each timepoint.

	Any LOS n=43	Confirmed LOS n=28	Clinical LOS n=15	No LOS n=76
CD45⁺ Leukocytes				
Day 1	6.03 (5.11, 7.12)	5.30 (3.65, 7.46)	7.32 (3.84, 11.8)	5.59 (4.94, 6.32)
Day 7	6.50 (5.59, 7.57)	5.30 (4.11, 7.49)	9.08 (6.95, 11.2)	5.90 (5.25, 6.62)
Day 14	6.20 (5.38, 7.15)	5.46 (4.68, 8.24)	6.41 (5.57, 7.54)	6.51 (5.84, 7.25)
Day 21	5.90 (5.15, 6.76)	5.68 (4.30, 6.81)	6.14 (4.84, 7.86)	5.54 (4.98, 6.16)
Day 28	5.43 (4.72, 6.23)	4.70 (3.65, 6.35)	7.54 (3.68, 8.51)	5.57 (5.00, 6.19)
Neutrophils				
Day 1	1.57 (1.30, 1.89)	2.00 (1.44, 2.78)	1.38 (0.88, 2.17)	2.26 (1.98, 2.59)
	36.6 (32.9, 40.3)	42.3 (37.0, 47.7)	33.7 (26.3, 41.1)	40.9 (38.2, 43.7)
Day 7	1.55 (1.29, 1.87)	1.51 (1.09, 2.10)	2.25 (1.43, 3.53)	1.91 (1.66, 2.19)
	34.4 (30.8, 38.1)	33.8 (28.4, 39.1)	43.3 (36.1, 50.5)	34.7 (31.9, 37.6)
Day 14	1.93 (1.59, 2.35)	2.06 (1.48, 2.87)	2.39 (1.50, 3.82)	2.22 (1.92, 2.57)
	38.8 (35.1, 42.5)	41.1 (35.9, 46.2)	42.4 (34.8, 49.9)	39.5 (36.7, 42.4)
Day 21	1.65 (1.35, 2.02)	1.82 (1.30, 2.56)	1.89 (1.19, 3.02)	1.79 (1.53, 2.10)
	34.0 (30.3, 37.6)	36.9 (31.8, 42.0)	36.4 (29.4, 43.4)	33.0 (30.1, 35.9)
Day 28	1.60 (1.28, 2.01)	1.66 (1.16, 2.37)	2.03 (1.25, 3.31)	1.46 (1.23, 1.73)
	34.4 (30.6, 38.1)	35.3 (30.0, 40.5)	40.8 (33.8, 47.9)	28.0 (25.1, 30.9)
Basophils				
Day 1	0.02 (0.02, 0.03)	0.02 (0.02, 0.03)	0.02 (0.02, 0.03)	0.02 (0.02, 0.03)
	0.41 (0.35, 0.49)	0.40 (0.32, 0.49)	0.36 (0.26, 0.49)	0.42 (0.37, 0.48)
Day 7	0.03 (0.03, 0.04)	0.03 (0.03, 0.04)	0.03 (0.02, 0.04)	0.04 (0.03, 0.05)
	0.56 (0.47, 0.67)	0.54 (0.43, 0.67)	0.49 (0.36, 0.67)	0.67 (0.59, 0.77)
Day 14	0.03 (0.03, 0.04)	0.03 (0.02, 0.04)	0.03 (0.02, 0.04)	0.04 (0.03, 0.05)
	0.52 (0.43, 0.62)	0.47 (0.38, 0.60)	0.49 (0.35, 0.69)	0.68 (0.59, 0.78)
Day 21	0.03 (0.02, 0.03)	0.03 (0.02, 0.03)	0.03 (0.02, 0.04)	0.03 (0.03, 0.04)
	0.49 (0.41, 0.59)	0.45 (0.36, 0.57)	0.47 (0.34, 0.64)	0.56 (0.48, 0.64)
Day 28	0.03 (0.02, 0.03)	0.03 (0.02, 0.03)	0.03 (0.02, 0.04)	0.03 (0.03, 0.03)
	0.48 (0.40, 0.58)	0.43 (0.33, 0.55)	0.47 (0.34, 0.66)	0.51 (0.44, 0.59)
Immature granulocytes				
Day 1	0.15 (0.12, 0.20)	0.15 (0.10, 0.20)	0.24 (0.15, 0.38)	0.16 (0.13, 0.20)
	2.62 (2.01, 3.43)	2.41 (1.73, 3.35)	3.99 (2.53, 6.27)	2.75 (2.25, 3.34)
Day 7	0.16 (0.12, 0.20)	0.17 (0.12, 0.25)	0.17 (0.11, 0.28)	0.15 (0.12, 0.18)
	2.65 (2.03, 3.47)	2.88 (2.03, 4.06)	2.88 (1.80, 4.61)	2.48 (2.02, 3.06)
Day 14	0.14 (0.10, 0.18)	0.16 (0.11, 0.23)	0.13 (0.08, 0.22)	0.09 (0.07, 0.11)
	2.32 (1.76, 3.07)	2.69 (1.88, 3.85)	2.20 (1.31, 3.71)	1.49 (1.21, 1.85)
Day 21	0.10 (0.07, 0.13)	0.11 (0.07, 0.16)	0.11 (0.06, 0.18)	0.07 (0.05, 0.08)
	1.62 (1.21, 2.16)	1.79 (1.22, 2.62)	1.76 (1.04, 2.96)	1.11 (0.88, 1.39)
Day 28	0.07 (0.05, 0.10)	0.07 (0.05, 0.11)	0.10 (0.06, 0.17)	0.06 (0.04, 0.07)
	1.22 (0.89, 1.68)	1.22 (0.81, 1.86)	1.65 (0.94, 2.89)	0.97 (0.75, 1.24)
Eosinophils				
Day 1	0.02 (0.01, 0.02)	0.01 (0.01, 0.02)	0.03 (0.02, 0.05)	0.02 (0.02, 0.03)
	0.26 (0.18, 0.37)	0.19 (0.12, 0.30)	0.48 (0.26, 0.90)	0.40 (0.31, 0.52)
Day 7	0.09 (0.06, 0.12)	0.08 (0.05, 0.12)	0.12 (0.06, 0.23)	0.12 (0.09, 0.16)
	1.46 (1.03, 2.08)	1.28 (0.79, 2.05)	1.98 (1.04, 3.77)	2.02 (1.55, 2.64)
Day 14	0.09 (0.06, 0.13)	0.08 (0.05, 0.13)	0.14 (0.07, 0.28)	0.09 (0.07, 0.11)
	1.54 (1.08, 2.19)	1.28 (0.78, 2.08)	2.26 (1.11, 4.59)	1.45 (1.11, 1.91)
Day 21	0.15 (0.10, 0.21)	0.13 (0.08, 0.22)	0.20 (0.10, 0.41)	0.16 (0.12, 0.22)
	2.53 (1.77, 3.63)	2.18 (1.30, 3.65)	3.33 (1.64, 6.76)	2.79 (2.10, 3.69)

Day 28	0.15 (0.10, 0.21) 2.47 (1.69, 3.62)	0.16 (0.09, 0.27) 2.60 (1.49, 4.53)	0.13 (0.06, 0.28) 2.19 (1.03, 4.66)	0.16 (0.12, 0.22) 2.75 (2.05, 3.70)
Classical monocytes				
Day 1	0.33 (0.25, 0.42) 5.53 (4.27, 7.17)	0.31 (0.24, 0.40) 5.13 (3.95, 6.66)	0.33 (0.23, 0.48) 5.50 (3.84, 7.86)	0.37 (0.31, 0.45) 6.32 (5.23, 7.64)
Day 7	0.45 (0.34, 0.59) 7.62 (5.83, 9.06)	0.42 (0.33, 0.55) 7.01 (5.43, 9.06)	0.46 (0.32, 0.65) 7.56 (5.34, 10.7)	0.43 (0.35, 0.53) 7.36 (6.00, 9.01)
Day 14	0.40 (0.30, 0.52) 6.73 (5.10, 8.87)	0.33 (0.26, 0.42) 5.43 (4.25, 6.94)	0.54 (0.38, 0.77) 8.92 (6.22, 12.8)	0.41 (0.33, 0.50) 6.88 (5.59, 8.48)
Day 21	0.42 (0.32, 0.56) 7.13 (5.38, 9.46)	0.35 (0.27, 0.44) 5.73 (4.51, 7.27)	0.56 (0.41, 0.78) 9.34 (6.73, 13.0)	0.36 (0.29, 0.45) 6.08 (4.90, 7.55)
Day 28	0.47 (0.35, 0.63) 7.89 (5.88, 10.6)	0.46 (0.36, 0.58) 7.53 (5.92, 9.59)	0.47 (0.34, 0.65) 7.74 (5.60, 10.7)	0.37 (0.29, 0.46) 6.20 (4.96, 7.74)
Non-classical monocytes				
Day 1	0.05 (0.04, 0.06) 0.77 (0.62, 0.96)	0.05 (0.04, 0.07) 0.80 (0.60, 1.08)	0.05 (0.03, 0.08) 0.85 (0.56, 1.28)	0.06 (0.05, 0.07) 1.06 (0.90, 1.24)
Day 7	0.05 (0.04, 0.06) 0.81 (0.66, 1.00)	0.05 (0.04, 0.06) 0.78 (0.59, 1.05)	0.06 (0.04, 0.09) 1.02 (0.69, 1.51)	0.06 (0.05, 0.07) 1.04 (0.88, 1.22)
Day 14	0.07 (0.05, 0.08) 1.10 (0.90, 1.35)	0.06 (0.05, 0.08) 1.02 (0.77, 1.35)	0.09 (0.06, 0.14) 1.57 (1.04, 2.35)	0.08 (0.07, 0.10) 1.43 (1.23, 1.68)
Day 21	0.08 (0.07, 0.10) 1.37 (1.13, 1.67)	0.08 (0.06, 0.11) 1.35 (1.03, 1.78)	0.10 (0.07, 0.15) 1.67 (1.15, 2.43)	0.07 (0.06, 0.09) 1.25 (1.07, 1.46)
Day 28	0.08 (0.07, 0.10) 1.36 (1.11, 1.67)	0.08 (0.06, 0.11) 1.40 (1.06, 1.85)	0.09 (0.06, 0.13) 1.53 (1.05, 2.23)	0.09 (0.07, 0.10) 1.46 (1.25, 1.71)
Non-cytotoxic T lymphocytes				
Day 1	0.88 (0.76, 1.01) 18.0 (15.3, 20.5)	0.71 (0.59, 0.86) 14.2 (11.2, 17.2)	0.93 (0.71, 1.20) 18.7 (14.6, 22.8)	0.98 (0.88, 1.08) 18.3 (16.5, 20.1)
Day 7	1.24 (1.08, 1.41) 24.4 (21.8, 26.9)	1.28 (1.07, 1.53) 24.6 (21.7, 27.4)	0.87 (0.68, 1.11) 17.8 (13.9, 21.7)	1.36 (1.23, 1.51) 25.6 (23.7, 27.5)
Day 14	1.14 (1.00, 1.30) 21.6 (19.0, 24.3)	1.01 (0.85, 1.20) 19.6 (16.8, 22.4)	1.05 (0.82, 1.36) 19.1 (15.1, 23.2)	1.25 (1.13, 1.38) 23.5 (21.5, 25.6)
Day 21	1.17 (1.03, 1.34) 22.3 (19.5, 25.2)	1.15 (0.95, 1.37) 21.7 (18.6, 24.7)	0.88 (0.68, 1.13) 16.9 (12.7, 21.1)	1.47 (1.33, 1.63) 27.7 (25.5, 29.9)
Day 28	1.25 (1.09, 1.45) 23.9 (20.8, 27.1)	1.16 (0.95, 1.41) 22.1 (18.6, 25.6)	1.02 (0.78, 1.34) 20.3 (15.6, 25.1)	1.59 (1.42, 1.78) 30.4 (27.9, 32.8)
Cytotoxic T/NK lymphocytes				
Day 1	0.03 (0.03, 0.04) 0.56 (0.44, 0.71)	0.03 (0.02, 0.04) 0.43 (0.31, 0.59)	0.04 (0.03, 0.07) 0.71 (0.46, 1.10)	0.03 (0.03, 0.04) 0.58 (0.48, 0.69)
Day 7	0.02 (0.02, 0.03) 0.37 (0.29, 0.46)	0.02 (0.01, 0.03) 0.33 (0.24, 0.46)	0.02 (0.01, 0.03) 0.36 (0.23, 0.55)	0.03 (0.02, 0.03) 0.47 (0.40, 0.57)
Day 14	0.03 (0.02, 0.04) 0.52 (0.41, 0.65)	0.03 (0.02, 0.04) 0.43 (0.31, 0.59)	0.04 (0.02, 0.06) 0.60 (0.38, 0.96)	0.03 (0.02, 0.04) 0.51 (0.42, 0.60)
Day 21	0.05 (0.04, 0.07) 0.89 (0.70, 1.12)	0.06 (0.04, 0.08) 0.94 (0.68, 1.29)	0.04 (0.02, 0.06) 0.62 (0.40, 0.96)	0.04 (0.03, 0.05) 0.66 (0.55, 0.79)
Day 28	0.06 (0.04, 0.07) 0.96 (0.74, 1.23)	0.06 (0.04, 0.08) 0.95 (0.68, 1.31)	0.05 (0.03, 0.07) 0.75 (0.48, 1.15)	0.05 (0.04, 0.06) 0.87 (0.72, 1.06)
B lymphocytes				
Day 1	0.17 (0.12, 0.24) 2.95 (2.11, 4.14)	0.17 (0.10, 0.27) 2.77 (1.70, 4.51)	0.15 (0.08, 0.29) 2.43 (1.25, 4.75)	0.18 (0.14, 0.24) 3.11 (2.42, 3.99)
Day 7	0.18 (0.13, 0.25) 3.08 (2.28, 4.16)	0.22 (0.15, 0.33) 3.65 (2.44, 5.46)	0.01 (0.06, 0.17) 1.65 (0.95, 2.86)	0.18 (0.14, 0.23) 3.06 (2.43, 3.85)
Day 14	0.25 (0.19, 0.33) 4.26 (3.21, 5.66)	0.23 (0.17, 0.32) 3.84 (2.77, 5.32)	0.23 (0.15, 0.37) 3.88 (2.43, 6.18)	0.22 (0.18, 0.27) 3.69 (2.97, 4.59)
Day 21	0.27 (0.21, 0.36) 4.64 (3.49, 6.17)	0.28 (0.21, 0.38) 4.70 (3.55, 6.22)	0.20 (0.14, 0.29) 3.30 (2.25, 4.85)	0.21 (0.17, 0.26) 3.54 (2.83, 4.42)

Day 28	0.27 (0.20, 0.37)	0.28 (0.21, 0.37)	0.21 (0.14, 0.30)	0.26 (0.20, 0.33)
	4.61 (3.35, 6.33)	4.58 (3.45, 6.08)	3.40 (2.32, 4.97)	4.32 (3.38, 5.54)

Supplementary Table 4. Adjusted mean (95% CI) percentage changes overtime in absolute counts and frequency percentages of total CD45⁺ leukocyte populations in preterm infants with and without LOS. Pairwise comparisons were corrected for ten multiple tests using the Bonferroni method to maintain an overall alpha error rate of 0.05.

	Any LOS		Confirmed LOS		Clinical LOS		No LOS	
	n=43	<i>P</i> value	n=28	<i>P</i> value	n=15	<i>P</i> value	n=76	<i>P</i> value
Neutrophils								
Day 7 vs Day 1	-1 (-22, 26)	1.000	-24 (-49, 11)	0.436	63 (-3, 175)	0.085	-16 (-30, 2)	0.113
Day 14 vs Day 1	23 (-5, 60)	0.236	3 (-31, 54)	1.000	74 (-1, 206)	0.062	-2 (-20, 20)	1.000
Day 21 vs Day 1	5 (-21, 40)	1.000	-9 (-40, 39)	1.000	38 (-23, 145)	1.000	-21 (-36, -1)	0.034
Day 28 vs Day 1	2 (-25, 40)	1.000	-17 (-47, 32)	1.000	48 (-21, 175)	0.791	-35 (-49, -17)	<0.001
Day 14 vs Day 7	24 (-3, 59)	0.127	36 (-7, 101)	0.241	6 (-38, 84)	1.000	17 (-4, 42)	0.248
Day 21 vs Day 7	6 (-18, 38)	1.000	20 (-19, 80)	1.000	-16 (-51, 46)	1.000	-6 (-23, 16)	1.000
Day 28 vs Day 7	3 (-22, 37)	1.000	10 (-29, 69)	1.000	-10 (-49, 62)	1.000	-23 (-39, -4)	0.009
Day 21 vs Day 14	-15 (-33, 9)	0.696	-12 (-40, 29)	1.000	-21 (-54, 37)	1.000	-19 (-34, -2)	0.019
Day 28 vs Day 14	-17 (-36, 8)	0.461	-20 (-46, 21)	1.000	-15 (-52, 50)	1.000	-34 (-46, -19)	<0.001
Day 28 vs Day 21	-3 (-24, 24)	1.000	-9 (-38, 34)	1.000	7 (-36, 81)	1.000	-19 (-33, -1)	0.028
Basophils								
Day 7 vs Day 1	36 (3, 79)	0.018	35 (-10, 104)	0.369	38 (-21, 139)	1.000	59 (28, 97)	<0.001
Day 14 vs Day 1	25 (-5, 65)	0.241	19 (-20, 79)	1.000	37 (-23, 144)	1.000	60 (29, 99)	<0.001
Day 21 vs Day 1	19 (-9, 57)	0.708	14 (-24, 72)	1.000	30 (-26, 127)	1.000	31 (6, 63)	0.004
Day 28 vs Day 1	16 (-12, 54)	1.000	8 (-29, 64)	1.000	32 (-25, 132)	1.000	19 (-4, 49)	0.223
Day 14 vs Day 7	-8 (-30, 22)	1.000	-12 (-41, 33)	1.000	-1 (-44, 77)	1.000	1 (-19, 25)	1.000
Day 21 vs Day 7	-12 (-33, 16)	1.000	-16 (-44, 27)	1.000	-6 (-46, 64)	1.000	-17 (-34, 3)	0.140
Day 28 vs Day 7	-14 (-35, 13)	1.000	-20 (-47, 21)	1.000	-4 (-45, 68)	1.000	-25 (-40, -6)	0.003
Day 21 vs Day 14	-4 (-28, 26)	1.000	-4 (-36, 43)	1.000	-5 (-46, 72)	1.000	-18 (-34, 2)	0.113
Day 28 vs Day 14	-7 (-30, 23)	1.000	-9 (-40, 37)	1.000	-3 (-46, 72)	1.000	-25 (-40, -7)	0.002
Day 28 vs Day 21	-3 (-26, 28)	1.000	-5 (-37, 43)	1.000	2 (-41, 77)	1.000	-9 (-27, 13)	1.000
Immature granulocytes								
Day 7 vs Day 1	1 (-36, 60)	1.000	20 (-36, 124)	1.000	-28 (-69, 69)	1.000	-10 (-37, 29)	1.000
Day 14 vs Day 1	-11 (-45, 43)	1.000	12 (-41, 111)	1.000	-45 (-78, 36)	0.650	-46 (-62, -21)	<0.001
Day 21 vs Day 1	-38 (-63, 2)	0.069	-26 (-61, 43)	1.000	-56 (-82, 8)	0.101	-60 (-73, -40)	<0.001
Day 28 vs Day 1	-53 (-73, -19)	0.001	-49 (-74, 1)	0.059	-59 (-84, 5)	0.078	-65 (-77, -46)	<0.001

Day 14 vs Day 7	-12 (-45, 39)	1.000	-6 (-50, 75)	1.000	-24 (-69, 86)	1.000	-40 (-58, -14)	0.001
Day 21 vs Day 7	-39 (-62, -2)	0.034	-38 (-67, 18)	0.368	-39 (-74, 45)	1.000	-55 (-69, -35)	<0.001
Day 28 vs Day 7	-54 (-72, -23)	<0.001	-57 (-78, -17)	0.003	-43 (-77, 40)	0.804	-61 (-74, -42)	<0.001
Day 21 vs Day 14	-30 (-56, 10)	0.264	-34 (-64, 24)	0.640	-20 (-67, 93)	1.000	-26 (-48, 7)	0.210
Day 28 vs Day 14	-47 (-67, -15)	0.002	-55 (-76, -13)	0.006	-25 (-70, 84)	1.000	-35 (-56, -6)	0.011
Day 28 vs Day 21	-24 (-52, 19)	0.866	-32 (-64, 29)	0.920	-6 (-60, 119)	1.000	-13 (-39, 25)	1.000

Eosinophils

Day 7 vs Day 1	461 (224, 870)	<0.001	573 (200, 1410)	<0.001	313 (38, 1130)	0.003	401 (227, 667)	<0.001
Day 14 vs Day 1	488 (233, 940)	<0.001	572 (197, 1420)	<0.001	371 (47, 1410)	0.002	260 (132, 459)	<0.001
Day 21 vs Day 1	868 (440, 1640)	<0.001	1050 (396, 2570)	<0.001	595 (120, 2100)	<0.001	591 (338, 990)	<0.001
Day 28 vs Day 1	845 (408, 1660)	<0.001	1270 (465, 3210)	<0.001	357 (38, 1410)	0.004	583 (323, 1000)	<0.001
Day 14 vs Day 7	5 (-40, 83)	1.000	0 (-56, 124)	1.000	14 (-64, 258)	1.000	-28 (-53, 11)	0.330
Day 21 vs Day 7	73 (-2, 204)	0.064	71 (-25, 289)	0.679	68 (-45, 416)	1.000	38 (-12, 115)	0.430
Day 28 vs Day 7	69 (-7, 205)	0.135	103 (-14, 381)	0.207	11 (-65, 251)	1.000	36 (-14, 116)	0.606
Day 21 vs Day 14	65 (-5, 186)	0.110	71 (-23, 281)	0.596	48 (-53, 362)	1.000	92 (24, 196)	<0.001
Day 28 vs Day 14	61 (-9, 185)	0.197	104 (-11, 367)	0.162	-3 (-70, 210)	1.000	89 (21, 196)	0.001
Day 28 vs Day 21	-2 (-44, 69)	1.000	19 (-47, 168)	1.000	-34 (-78, 96)	1.000	-1 (-36, 52)	1.000

Classical monocytes

Day 7 vs Day 1	38 (-1, 92)	0.064	37 (-15, 120)	0.670	38 (-28, 163)	1.000	16 (-10, 51)	1.000
Day 14 vs Day 1	22 (-13, 70)	1.000	6 (-34, 70)	1.000	62 (-18, 220)	0.446	9 (-16, 42)	1.000
Day 21 vs Day 1	29 (-8, 81)	0.343	12 (-31, 81)	1.000	70 (-12, 229)	0.238	-4 (-26, 26)	1.000
Day 28 vs Day 1	43 (1, 102)	0.042	47 (-10, 140)	0.289	41 (-28, 175)	1.000	-2 (-25, 29)	1.000
Day 14 vs Day 7	-12 (-37, 23)	1.000	-23 (-52, 25)	1.000	18 (-40, 132)	1.000	-6 (-28, 22)	1.000
Day 21 vs Day 7	-6 (-33, 31)	1.000	-18 (-50, 32)	1.000	24 (-36, 138)	1.000	-17 (-37, 8)	0.455
Day 28 vs Day 7	4 (-27, 46)	1.000	7 (-34, 76)	1.000	2 (-47, 98)	1.000	-16 (-36, 10)	0.733
Day 21 vs Day 14	6 (-24, 48)	1.000	5 (-34, 69)	1.000	5 (-47, 105)	1.000	-12 (-32, 15)	1.000
Day 28 vs Day 14	17 (-16, 65)	1.000	39 (-14, 125)	0.569	-13 (-56, 71)	1.000	-10 (-31, 17)	1.000
Day 28 vs Day 21	11 (-21, 54)	1.000	32 (-18, 112)	1.000	-17 (-57, 58)	1.000	2 (-22, 32)	1.000

Non-classical monocytes

Day 7 vs Day 1	5 (-25, 47)	1.000	-2 (-41, 61)	1.000	20 (-39, 136)	1.000	-2 (-24, 27)	1.000
Day 14 vs Day 1	43 (1, 103)	0.039	27 (-23, 111)	1.000	84 (-10, 277)	0.163	36 (4, 77)	0.014
Day 21 vs Day 1	78 (24, 155)	<0.001	69 (1, 182)	0.044	96 (-3, 297)	0.072	18 (-11, 57)	0.953

Day 28 vs Day 1	77 (20, 160)	<0.001	75 (2, 198)	0.035	80 (-13, 272)	0.230	38 (3, 86)	0.021
Day 14 vs Day 7	36 (-3, 92)	0.107	30 (-21, 115)	1.000	53 (-24, 212)	0.894	38 (6, 81)	0.006
Day 21 vs Day 7	69 (20, 139)	<0.001	73 (4, 187)	0.025	63 (-18, 226)	0.460	21 (-8, 59)	0.565
Day 28 vs Day 7	68 (17, 143)	0.001	79 (6, 203)	0.019	50 (-26, 204)	1.000	41 (6, 88)	0.007
Day 21 vs Day 14	24 (-11, 74)	0.712	32 (-19, 117)	1.000	6 (-47, 115)	1.000	-13 (-33, 14)	1.000
Day 28 vs Day 14	23 (-13, 75)	0.925	37 (-18, 128)	0.825	-2 (-52, 100)	1.000	2 (-22, 34)	1.000
Day 28 vs Day 21	-1 (-29, 39)	1.000	4 (-37, 71)	1.000	-8 (-53, 80)	1.000	17 (-10, 53)	0.962

Non-cytotoxic T lymphocytes

Day 7 vs Day 1	41 (11, 80)	0.001	79 (36, 137)	<0.001	-6 (-36, 36)	1.000	39 (15, 68)	<0.001
Day 14 vs Day 1	30 (1, 66)	0.033	41 (5, 90)	0.009	14 (-25, 72)	1.000	22 (0, 48)	0.041
Day 21 vs Day 1	34 (4, 72)	0.011	61 (17, 121)	<0.001	-5 (-39, 47)	1.000	41 (16, 72)	<0.001
Day 28 vs Day 1	43 (11, 86)	0.001	62 (13, 132)	0.002	11 (-32, 80)	1.000	63 (33, 99)	<0.001
Day 14 vs Day 7	-8 (-28, 18)	1.000	-21 (-40, 4)	0.172	21 (-18, 80)	1.000	-13 (-28, 6)	0.493
Day 21 vs Day 7	-5 (-26, 21)	1.000	-10 (-33, 21)	1.000	1 (-32, 51)	1.000	1 (-17, 23)	1.000
Day 28 vs Day 7	2 (-21, 31)	1.000	-10 (-35, 25)	1.000	18 (-24, 83)	1.000	17 (-4, 43)	0.284
Day 21 vs Day 14	3 (-19, 32)	1.000	14 (-14, 50)	1.000	-17 (-44, 24)	1.000	16 (-4, 41)	0.303
Day 28 vs Day 14	11 (-14, 42)	1.000	14 (-15, 54)	1.000	-3 (-36, 48)	1.000	34 (10, 63)	<0.001
Day 28 vs Day 21	7 (-16, 37)	1.000	10 (-24, 33)	1.000	17 (-20, 70)	1.000	15 (-5, 39)	0.391

Cytotoxic T/NK lymphocytes

Day 7 vs Day 1	-34 (-56, -2)	0.029	-22 (-55, 35)	1.000	-50 (-76, 6)	0.093	-18 (-39, 11)	0.683
Day 14 vs Day 1	-8 (-39, 39)	1.000	-1 (-43, 71)	1.000	-15 (-61, 85)	1.000	-13 (-36, 20)	1.000
Day 21 vs Day 1	59 (3, 144)	0.026	118 (26, 277)	0.001	-13 (-59, 84)	1.000	13 (-19, 59)	1.000
Day 28 vs Day 1	71 (7, 171)	0.012	120 (26, 282)	0.001	5 (-50, 122)	1.000	51 (6, 116)	0.011
Day 14 vs Day 7	40 (-6, 109)	0.175	27 (-27, 122)	1.000	70 (-22, 272)	0.580	7 (-22, 46)	1.000
Day 21 vs Day 7	141 (60, 262)	<0.001	180 (61, 387)	<0.001	74 (-18, 269)	0.390	38 (0, 91)	0.051
Day 28 vs Day 7	159 (68, 301)	<0.001	182 (61, 395)	<0.001	110 (-1, 346)	0.057	85 (31, 159)	<0.001
Day 21 vs Day 14	72 (16, 155)	0.001	120 (27, 281)	0.001	2 (-53, 124)	1.000	30 (-5, 78)	0.199
Day 28 vs Day 14	85 (22, 180)	<0.001	122 (27, 287)	0.001	24 (-43, 170)	1.000	73 (25, 139)	<0.001
Day 28 vs Day 21	18 (-27, 60)	1.000	1 (-42, 76)	1.000	21 (-43, 155)	1.000	33 (-2, 82)	0.092

B lymphocytes

Day 7 vs Day 1	4 (-33, 63)	1.000	32 (-20, 118)	1.000	-32 (-66, 34)	1.000	-2 (-31, 39)	1.000
Day 14 vs Day 1	44 (-12, 137)	0.389	39 (-22, 146)	1.000	59 (-29, 256)	1.000	19 (-19, 74)	1.000

Day 21 vs Day 1	57 (-11, 176)	0.246	70 (-14, 234)	0.281	36 (-46, 243)	1.000	14 (-26, 76)	1.000
Day 28 vs Day 1	56 (-19, 199)	0.559	65 (-26, 270)	0.794	40 (-53, 319)	1.000	39 (-16, 129)	0.641
Day 14 vs Day 7	38 (-12, 118)	0.447	5 (-36, 74)	1.000	135 (16, 376)	0.007	21 (-15, 72)	1.000
Day 21 vs Day 7	51 (-8, 147)	0.205	29 (-28, 129)	1.000	100 (-9, 338)	0.129	16 (-22, 71)	1.000
Day 28 vs Day 7	50 (-15, 165)	0.475	25 (-37, 149)	1.000	106 (-19, 421)	0.289	41 (-9, 120)	0.282
Day 21 vs Day 14	9 (-31, 71)	1.000	22 (-25, 101)	1.000	-15 (-58, 72)	1.000	-4 (-33, 37)	1.000
Day 28 vs Day 14	8 (-35, 79)	1.000	19 (-33, 113)	1.000	-12 (-61, 96)	1.000	17 (-21, 73)	1.000
Day 28 vs Day 21	-1 (-37, 56)	1.000	-3 (-41, 61)	1.000	3 (-48, 102)	1.000	22 (-14, 74)	1.000
