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Assessment of systemic circulation using ultrasound Doppler in late onset neonatal sepsis and its clinical correlation: an observational study

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Abstract

Objectives To measure the Doppler velocimetry parameters in the anterior cerebral artery (ACA), superior mesenteric artery (SMA), and main renal artery (RA) in neonates with late-onset sepsis and correlate it with associated clinical morbidities.

Methodology Prospective observational study carried out at a tertiary-level neonatal intensive care unit in India in 2022, enrolling 20 neonates with late-onset neonatal sepsis (LONS). Baseline characteristics and sepsis parameters obtained. Serial ultrasound performed on days 1, 3, and 7 from the day of clinical sepsis in the ACA, SMA, and RA and velocimetry measurements obtained. The findings were compared with 20 gestational age (GA) matched neonates in the control arm.

Results The mean GA of neonates with LONS was 31.03 ± 2.79 weeks and their mean birthweight was 1474 ± 509.99 g. The peak systolic velocity, resistive and pulsatility indices were significantly higher in ACA, SMA, and RA and the end-diastolic velocity was significantly lower in ACA and RA ($P < 0.05$) in LONS. The incidences of intraventricular hemorrhage (IVH), necrotising enterocolitis (NEC), and acute kidney injury (AKI) in neonates with LONS were 45%, 50%, and 10% respectively. A subgroup analysis of the Doppler velocimetry parameters in the neonates with LONS and for neonates with and without clinical outcomes did not suggest a significant difference.

Conclusion LONS is associated with alterations in cerebral, splanchnic, and renal perfusion seen as abnormal blood flow velocimetry and vascular resistance which may predispose to IVH, NEC, and AKI.

Keywords Late-onset sepsis · Ultrasound Doppler · Neonate · Systemic circulation

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Introduction

Worldwide, sepsis is one of the commonest causes of neo-natal morbidity and mortality [1, 2] and is characterised by systemic signs of infection, accompanied with bacteremia [3]. Owing to higher antenatal antibiotic exposure, diagnostic and culture yield of early onset neonatal sepsis is low and unreliable, specially in the setting of non-specific clinical features in low-middle income setting [4]. Late-onset neo-natal sepsis (LONS) is defined when clinical sepsis occurs in a neonate beyond 72 h of life [5]. Hemodynamics in LONS is multifactorial, complex in nature and incompletely understood. LONS in our country is mainly caused by gram nega-tive organisms with high mortality rate [1]. Understanding the functional characteristics of neonatal hemodynamics may prove to be significant in the development of thera-peutic targets and interventions [6]. Circulating cytokines, interleukins and endogenous catecholamines secreted in

response to sepsis-related endotoxemia via various inflammatory pathways directly influence tissue perfusion and systemic circulation [7]. The assessment of systemic circulation is done indirectly by evaluating surrogate parameters such as capillary refill time, central-peripheral temperature difference, heart rate, urine output and systemic blood pressure [6]. The systemic inflammatory response leading to endotoxemia and vasoreactivity directly influences the cerebral and systemic vascular resistance [7–9], thus altering the blood flow. Therefore, a direct assessment of arterial Doppler flow velocities can prove to be helpful for monitoring organ perfusion, to guide treatment strategies as well as monitor for associated co-morbidities such as intraventricular hemorrhage (IVH), necrotising enterocolitis (NEC) and acute kidney injury (AKI).

The focus of the present study is to utilise bedside ultrasound to observe the systemic blood flow in LONS and to know the hemodynamic changes in the vascular system due to systemic inflammatory response. Color Doppler sonography enables visualisation of blood flow, non-invasive evaluation of vascular resistance (by measuring resistance indices) and measurement of blood flow velocities [10].

There is limited data for changes in organ perfusion in neonatal sepsis and detailed studies for evaluation of cerebral and systemic blood flow parameters in LONS are lacking in view of higher incidence of gram negative sepsis with non-specific diagnosis of early onset sepsis. The primary aim of this study was to document the pattern of systemic (cerebral, splanchnic and renal) circulation in LONS by Doppler ultrasonography and correlate it clinically with the incidence of IVH, NEC, and AKI in the study population.

Methods

A single center prospective observational study was conducted at a tertiary-level neonatal intensive care unit (NICU) in Western India. The recruitment began in January 2022 until June 2022 after obtaining approval from the institutional ethics committee. All neonates (> 26 weeks of gestation) admitted during the study period and diagnosed with LONS (> 72 h of life) were included and further sub-classified into:

1. Clinical sepsis is defined as the presence of two or more of the following categories of clinical signs: temperature instability, haemodynamic instability, metabolic derangement, respiratory distress, feed intolerance or neurological symptoms.
2. Laboratory proven sepsis is defined as two or more of the following hematological parameters:

   - C reactive protein (CRP) more than equal to 6 mg/L

   - Thrombocytopenia (platelet count $< 100,000/mm^3$)
   - Neutropenia with absolute neutrophil count (ANC) cut offs as per Manroe chart [11] (term babies), Mouzinho chart [12] (preterm babies)

3. Culture positive sepsis

Neonates with early onset neonatal sepsis (< 72 h of life), perinatal asphyxia, congenital heart disease, congenital malformations and genetic syndromes were excluded. Informed written consent was obtained from the relatives prior to enrolment in the study. Gestational age matched neonates, > 72 h old, born during the study period with no evidence of sepsis were taken as the control group.

Baseline maternal characteristics (parity, antenatal steroid cover, mode of delivery) and baseline neonatal characteristics (gestational age, birthweight, sex, 5 min APGAR score, cord clamping detail) were noted. Sepsis parameters such as c-reactive protein, total leukocyte count, absolute neutrophil count and platelet count were recorded and clinical details noted.

The enrolled neonates were evaluated using bedside ultrasound Doppler on day 1, day 3 and day 7 from the day of clinical sepsis. The assessment was performed by a senior neonatal fellow with at least 6 months of experience in the NICU and trained in neonatal ultrasonography and echocardiography under the supervision of a senior clinician. The procedure was performed in a quiet setting with neonates in supine and lateral decubitus position. No sedation was used for the procedure and feeding was ongoing as per the neonate's clinical status, regardless of the time of ultrasound scan. Ultrasound was performed using Philips Affinity 50G (Koninklijke Philips, Nevada, USA) machine using a 8–12 MHz linear transducer. The brain, abdomen, and kidneys were scanned and color flow mapping was performed to image blood flow in the anterior cerebral artery (ACA), superior mesenteric artery (SMA), and main renal artery (RA). The flow velocity waveforms were obtained at an optimal insonating angle ($< 50°$). Values of peak systolic velocity (PSV), end-diastolic velocity (EDV), resistive index (RI) and pulsatility index (PI) were recorded (Fig. 1). Three measurements of the arteries were obtained each time and a calculated mean of three values was used.

Statistical analysis

Qualitative data was interpreted as frequencies and percentages, whereas quantitative data was interpreted as mean, standard deviation, or median and range. For the comparison of the means of 2 independent groups, an unpaired t-test was used. For the comparison of the means of 3 independent groups, the ANOVA (analysis of variance) test was applied.
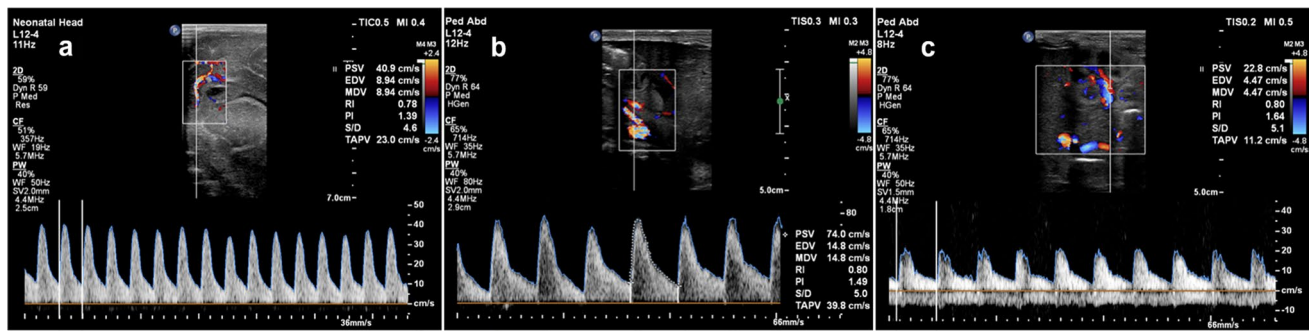
Fig. 1 Doppler ultrasonography of blood flow parameters in **a** anterior cerebral artery **b** superior mesenteric artery and **c** main renal artery

Table 1 Baseline characteristics of the study cohort

	Cases N (%)	Controls N (%)	P-value
Maternal characteristics ($n=20$)			
Parity			
Primigravida	13 (65%)	12 (60%)	0.75
Multigravida	7 (35%)	8 (40%)	0.75
Complete Steroid cover	12 (60%)	10 (50%)	0.53
Mode of delivery			
Vaginal delivery	5 (25%)	4 (20%)	0.71
Caesarean section	15 (75%)	16 (80%)	0.71
Neonatal characteristics ($n=20$)			
Males	9 (45%)	10 (50%)	0.75
Mean gestational age	31.03 ± 2.79	32.8 ± 3.05	0.063
Mean birth weight	1474 ± 509.99	1628 ± 603.41	0.388
Mean 5 min APGAR	7.6 ± 0.6	7.95 ± 0.89	0.153
Delayed cord clamping	12 (60%)	11 (55%)	0.75

The data was analysed using SPSS version 25.0. P value of < 0.05 was considered statistically significant.

Results

Over a period of six months, a total of 682 patients were admitted to NICU and 27 were diagnosed with late-onset neonatal sepsis. Twenty babies matching the inclusion criteria were enrolled and compared with 20 gestational age-matched neonates without sepsis and not requiring inotrope.

The baseline characteristics of the study population were comparable (Table 1). The mean gestational age of the LONS group was 31.03 ± 2.79 weeks and of controls was 32.8 ± 3.05 weeks. The mean birthweight was 1474 ± 509.99 and 1628 ± 603.41, respectively, for the case and control groups. Most of the neonates in both groups were delivered to multigravida mothers and the mode of delivery was lower segment caesarean section. It was observed that 40% of mothers in the case cohort and 50% in the control group did not receive a complete course of steroids. Majority of neonates in both the groups underwent delayed cord clamping and this proportion was 60% and 55% for cases and controls. Mean 5-min APGAR scores were 7.6 ± 0.6 and 7.95 ± 0.89 for both groups.

Out of 20 cases, 3 were diagnosed with clinical sepsis, 7 with laboratory proven sepsis and 10 had culture-positive sepsis. Mean gestational age across the three groups was 32.4 ± 2.42, 31.86 ± 1.86, and 30.04 ± 3.24 weeks, respectively, and mean birthweight was 1670 ± 487.54, 1637 ± 467.11, and 1301 ± 432.95 g, respectively. The mean value of c-reactive protein was 1.00 mg/L in clinical sepsis, 45.57 ± 17.38 mg/L in lab proven sepsis and 61.6 ± 30.39 mg/L in culture-positive sepsis. Mean absolute neutrophil counts observed for these three sub-categories were 4666 ± 882, 3185 ± 896, and 1625 ± 592 /mm3, respectively. The median [IQR] value of platelet count for clinical sepsis was 2,14,000 /mm^3 and for lab and culture positive sepsis, were 98,000 [1, 13] and 14,500 [1–9, 9–17] /mm^3, respectively. Only one of the neonates in the case cohort required inotrope (milrinone) for biventricular dysfunction and high pulmonary pressures.

Upon comparison, it was observed that the values of ACA-PSV measured on days 1, 3, and 7 of diagnosis in the case-cohort were higher as compared to controls ($P < 0.05$). Similarly, SMA-PSV ($P > 0.05$) and RA-PSV ($P < 0.05$) values were also found to be higher in LONS. EDV was measured to be lower in sepsis with statistical significance in ACA and RA. RI, which is a marker of arterial impedance, was found to be higher in ACA and RA in sepsis ($P < 0.05$). Similarly, PI values were also elevated for the case-cohort in all three arteries and were statistically significant in ACA and RA (Table 2). In the LONS group, 45% of neonates in the case-cohort were diagnosed with IVH, 50% had NEC, and 10% had AKI. Compared to this, 10% of the neonates in the control group developed

Table 2 Doppler ultrasonography blood flow parameters in the study population

	Cases ($n=20$)	Controls ($n=20$)	P-value
ACA PSV			
Day 1 of diagnosis	46.88 ± 17.69	31.92 ± 9.59	**0.002**
Day 3 of diagnosis	50.61 ± 17.07	33.14 ± 10.31	**0.0004**
Day 7 of diagnosis	51.71 ± 12.55	34.94 ± 12.23	**0.0001**
ACA EDV			
Day 1 of diagnosis	6.21 ± 2.23	8.16 ± 2.6	**0.0151**
Day 3 of diagnosis	6.25 ± 2.32	8.34 ± 2.28	**0.0066**
Day 7 of diagnosis	6.58 ± 1.91	9.27 ± 3.46	**0.0042**
ACA RI			
Day 1 of diagnosis	0.82 ± 0.08	0.73 ± 0.06	**0.0003**
Day 3 of diagnosis	0.83 ± 0.07	0.73 ± 0.08	**0.0002**
Day 7 of diagnosis	0.84 ± 0.06	0.73 ± 0.06	**< 0.0001**
ACA PI			
Day 1 of diagnosis	1.74 ± 0.35	1.41 ± 0.25	**0.0015**
Day 3 of diagnosis	1.8 ± 0.39	1.37 ± 0.27	**0.0002**
Day 7 of diagnosis	1.95 ± 0.42	1.35 ± 0.24	**< 0.0001**
SMA PSV			
Day 1 of diagnosis	75.96 ± 32.03	69.53 ± 16.57	0.4302
Day 3 of diagnosis	74.56 ± 27.47	70.09 ± 16.64	0.5374
Day 7 of diagnosis	81.79 ± 29.04	69.74 ± 18.36	0.1251
SMA EDV			
Day 1 of diagnosis	18.78 ± 6.83	17.08 ± 3.08	0.3167
Day 3 of diagnosis	17.29 ± 5.53	16.69 ± 3.67	0.6883
Day 7 of diagnosis	16.84 ± 5.95	17.33 ± 3.5	0.7526
SMA RI			
Day 1 of diagnosis	0.71 ± 0.15	0.73 ± 0.07	0.5921
Day 3 of diagnosis	0.76 ± 0.1	0.74 ± 0.08	0.4892
Day 7 of diagnosis	0.76 ± 0.1	0.74 ± 0.07	0.4682
SMA PI			
Day 1 of diagnosis	1.91 ± 0.72	1.78 ± 0.53	0.5194
Day 3 of diagnosis	2.11 ± 0.64	1.77 ± 0.5	0.0689
Day 7 of diagnosis	2.16 ± 0.59	1.76 ± 0.42	**0.0181**
RA PSV			
Day 1 of diagnosis	65.9 ± 24.96	40.83 ± 19.29	**0.001**
Day 3 of diagnosis	66.34 ± 27.25	42.8 ± 19.22	**0.0031**
Day 7 of diagnosis	70.17 ± 25.68	41.83 ± 17.07	**0.0002**
RA EDV			
Day 1 of diagnosis	3.71 ± 2.42	6.36 ± 1.19	**0.0001**
Day 3 of diagnosis	3.67 ± 2.46	7.1 ± 1.22	**< 0.0001**
Day 7 of diagnosis	3.38 ± 2.07	6.41 ± 1.12	**< 0.0001**
RA RI			
Day 1 of diagnosis	0.92 ± 0.12	0.82 ± 0.09	**0.005**
Day 3 of diagnosis	0.94 ± 0.12	0.81 ± 0.09	**0.0004**
Day 7 of diagnosis	0.93 ± 0.11	0.82 ± 0.08	**0.0009**
RA PI			
Day 1 of diagnosis	2.27 ± 0.62	1.91 ± 0.53	0.0557
Day 3 of diagnosis	2.42 ± 0.78	1.96 ± 0.54	**0.0365**
Day 7 of diagnosis	2.45 ± 0.8	1.87 ± 0.5	**0.0091**

The statistically significant outcomes with *P* values <0.05 have been highlighted in bold

ACA anterior cerebral artery, *PSV* peak systolic velocity, *EDV* end diastolic velocity, *RI* resistive index, *PI* pulsatility index, *SMA* superior mesenteric artery, *RA* renal artery
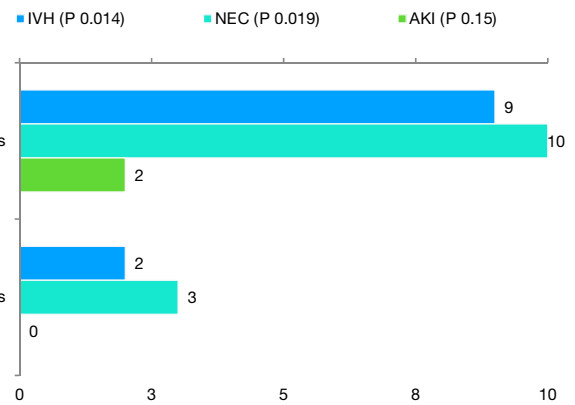
Fig. 2 Number of neonates with associated intraventricular hemorrhage (IVH), necrotising enterocolitis (NEC) and acute kidney injury (AKI) in the study population

IVH ($P < 0.05$), 15% had NEC ($P < 0.05$) and no neonate was diagnosed with AKI (P *P*.15) (Fig. 2).

A comparison of the values of PSV, EDV, RI, and PI measured in the three arteries on days 1, 3, and 7 of diagnosis of late onset neonatal sepsis was also done between the groups—clinical sepsis, laboratory proven sepsis and culture positive sepsis. However, a statistically significant difference could not be drawn from these values (Table 3).

An analysis of Doppler velocimetry parameters with clinical outcomes was also performed (Table 4). It was observed that PSV, EDV and PI were lower in neonates with IVH with RI value equivalent to those without IVH. Difference in the parameters in SMA between neonates with and without NEC was variable and a statistically significant result could only be obtained for PI. While PSV for neonates with AKI was higher ($P < 0.05$), EDV and PI were lower. For most of the outcomes, a statistically siginificant difference could not be drawn.

Discussion

Hemodynamic, along with cardiovascular disturbances, are commonly found in neonatal sepsis in both term and preterm babies [1]. While the studies on cardiovascular dysfunction utilising targeted neonatal echocardiography are plenty, the evidence for abnormal blood flow and organ perfusion is lacking. Some of the existing studies have evaluated Doppler blood flow velocimetries in neonates with early onset sepsis and have found significant difference in cerebral and splanchnic blood flow [13–15]. It has been established that the pathophysiology behind neonatal sepsis causes a hemodynamic disturbance; however, specific parameters for monitoring the same have not been isolated and elaborated upon [6]. Through this study, we evaluated the changes in blood flow and organ perfusion of

Table 3 Doppler ultrasonography blood flow parameters in neonates with sepsis

	Clinical sepsis (3)	Lab proven sepsis (7)	Culture positive sepsis (10)	P-value
ACA PSV				
Day 1 of diagnosis	48.53 ± 6.52	43.74 ± 10.1	56.58 ± 9.24	0.858
Day 3 of diagnosis	49.47 ± 8.17	45.46 ± 8.41	54.56 ± 8.32	0.578
Day 7 of diagnosis	49.47 ± 7.64	45.62 ± 5.66	52.5 ± 3.23	0.944
ACA EDV				
Day 1 of diagnosis	4.53 ± 1.01	6.76 ± 1.46	6.42 ± 1.36	0.361
Day 3 of diagnosis	4.73 ± 1.59	7.54 ± 1.72	5.8 ± 1.9	0.148
Day 7 of diagnosis	6.7 ± 1.82	8.24 ± 1.05	5.61 ± 1.25	**0.034**
ACA RI				
Day 1 of diagnosis	0.88 ± 0.04	0.79 ± 0.09	0.82 ± 0.08	0.293
Day 3 of diagnosis	0.83 ± 0.01	0.82 ± 0.09	0.84 ± 0.06	0.742
Day 7 of diagnosis	0.85 ± 0.02	0.81 ± 0.09	0.85 ± 0.05	0.521
ACA PI				
Day 1 of diagnosis	1.96 ± 0.45	1.96 ± 0.38	1.59 ± 0.3	0.160
Day 3 of diagnosis	1.88 ± 0.02	1.77 ± 0.5	1.8 ± 0.38	0.921
Day 7 of diagnosis	2.23 ± 0.41	2.08 ± 0.35	1.77 ± 0.42	0.194
SMA PSV				
Day 1 of diagnosis	99.67 ± 13.41	79.26 ± 19.54	66.54 ± 10.27	0.289
Day 3 of diagnosis	86.33 ± 16.08	80.09 ± 18.2	67.16 ± 18.38	0.483
Day 7 of diagnosis	94 ± 20.2	86.8 ± 13.96	74.94 ± 19.19	0.585
SMA EDV				
Day 1 of diagnosis	11.1 ± 2.39	16.93 ± 1.71	16.38 ± 1.1	0.431
Day 3 of diagnosis	10.8 ± 2.3	19.4 ± 1.46	17.76 ± 2.54	0.587
Day 7 of diagnosis	11.3 ± 2.3	19 ± 2.17	18.3 ± 2.88	0.713
SMA RI				
Day 1 of diagnosis	0.81 ± 0.15	0.75 ± 0.16	0.66 ± 0.13	0.237
Day 3 of diagnosis	0.74 ± 0.11	0.78 ± 0.12	0.65 ± 0.1	0.801
Day 7 of diagnosis	0.76 ± 0.09	0.73 ± 0.1	0.69 ± 0.1	0.905
SMA PI				
Day 1 of diagnosis	1.96 ± 0.73	2.19 ± 0.94	1.99 ± 0.51	0.381
Day 3 of diagnosis	1.82 ± 0.34	2.24 ± 0.86	2.11 ± 0.55	0.665
Day 7 of diagnosis	1.71 ± 0.51	2 ± 0.49	2.17 ± 0.47	0.370
RA PSV				
Day 1 of diagnosis	88.43 ± 9.03	56.11 ± 13.9	65.98 ± 8.93	0.175
Day 3 of diagnosis	77.23 ± 8.85	60.14 ± 12.74	67.4 ± 12.76	0.676
Day 7 of diagnosis	84 ± 6.08	66.04 ± 9.03	69.74 ± 10.51	0.440
RA EDV				
Day 1 of diagnosis	2.36 ± 0.19	4 ± 0.14	5.31 ± 0.63	0.819
Day 3 of diagnosis	2.86 ± 0.35	4.34 ± 0.9	4.89 ± 0.45	0.833
Day 7 of diagnosis	3.23 ± 0.4	3.35 ± 0.15	4.41 ± 0.61	0.777
RA RI				
Day 1 of diagnosis	1.04 ± 0.1	0.93 ± 0.12	0.87 ± 0.11	0.101
Day 3 of diagnosis	0.95 ± 0.05	0.98 ± 0.14	0.91 ± 0.12	0.551
Day 7 of diagnosis	0.95 ± 0.02	0.92 ± 0.12	0.9 ± 0.08	0.909
RA PI				
Day 1 of diagnosis	2.43 ± 0.5	2.65 ± 0.73	2.01 ± 0.48	0.117
Day 3 of diagnosis	2.22 ± 0.45	2.79 ± 0.86	2.26 ± 0.79	0.394
Day 7 of diagnosis	2.18 ± 0.42	3.29 ± 0.81	2.16 ± 0.66	**0.038**

The statistically significant outcomes with P values <0.05 have been highlighted in bold

ACA anterior cerebral artery, *PSV* peak systolic velocity, *EDV* end diastolic velocity, *RI* resistive index, *PI* pulsatility index, *SMA* superior mesenteric artery, *RA* renal artery

Table 4 Doppler ultrasonography blood flow parameters in neonates with and without clinical outcomes

	IVH (n=9)	No IVH (n=11)	P-value
Mean ACA PSV			
Day 1 of diagnosis	42.6 ± 22.71	50.38 ± 12.33	0.34
Day 3 of diagnosis	45.31 ± 18.71	54.95 ± 15.08	0.21
Day 7 of diagnosis	46.96 ± 13.23	55.92 ± 10.92	0.11
Mean ACA EDV			
Day 1 of diagnosis	5.79 ± 2.17	6.55 ± 2.32	0.46
Day 3 of diagnosis	6.12 ± 2.21	6.35 ± 2.51	0.83
Day 7 of diagnosis	6.65 ± 1.76	6.51 ± 2.13	0.87
Mean ACA RI			
Day 1 of diagnosis	0.82 ± 0.08	0.82 ± 0.08	1
Day 3 of diagnosis	0.82 ± 0.06	0.84 ± 0.07	0.51
Day 7 of diagnosis	0.83 ± 0.08	0.85 ± 0.04	0.47
Mean ACA PI			
Day 1 of diagnosis	1.56 ± 0.34	1.88 ± 0.3	**0.038**
Day 3 of diagnosis	1.66 ± 0.51	1.91 ± 0.21	0.15
Day 7 of diagnosis	1.82 ± 0.46	2.06 ± 0.38	0.22
	NEC (n=10)	No NEC (n=10)	P-value
Mean SMA PSV			
Day 1 of diagnosis	74.41 ± 37.42	77.51 ± 27.58	0.84
Day 3 of diagnosis	75.15 ± 34.78	73.97 ± 19.56	0.93
Day 7 of diagnosis	88.29 ± 39.7	77.25 ± 19.82	0.44
Mean SMA EDV			
Day 1 of diagnosis	18.42 ± 6.93	19.14 ± 7.08	0.82
Day 3 of diagnosis	18.76 ± 6.99	15.82 ± 3.3	0.24
Day 7 of diagnosis	18.3 ± 7.66	15.37 ± 3.37	0.28
Mean SMA RI			
Day 1 of diagnosis	0.74 ± 0.16	0.68 ± 0.14	0.38
Day 3 of diagnosis	0.78 ± 0.11	0.74 ± 0.09	0.39
Day 7 of diagnosis	0.78 ± 0.1	0.74 ± 0.1	0.38
Mean SMA PI			
Day 1 of diagnosis	2.07 ± 0.66	1.75 ± 0.77	0.33
Day 3 of diagnosis	2.41 ± 0.7	1.82 ± 0.42	**0.03**
Day 7 of diagnosis	2.44 ± 0.59	1.88 ± 0.45	**0.028**
	AKI (n=2)	No AKI (n=18)	P-value
Mean RA PSV			
Day 1 of diagnosis	100 ± 14.14	62.11 ± 23.07	**0.037**
Day 3 of diagnosis	109 ± 15.56	61.59 ± 24.04	**0.015**
Day 7 of diagnosis	99 ± 15.56	66.85 ± 24.77	0.093
Mean RA EDV			
Day 1 of diagnosis	1.95 ± 0.35	3.91 ± 2.48	0.29
Day 3 of diagnosis	2.89 ± 1.26	3.76 ± 2.57	0.71
Day 7 of diagnosis	2.7 ± 1.14	3.46 ± 2.16	0.64
Mean RA RI			
Day 1 of diagnosis	0.93 ± 0.07	0.92 ± 0.13	0.92
Day 3 of diagnosis	0.93 ± 0.08	0.94 ± 0.13	0.92
Day 7 of diagnosis	0.91 ± 0.07	0.94 ± 0.11	0.71
Mean RA PI			
Day 1 of diagnosis	2.15 ± 0.07	2.29 ± 0.65	0.77
Day 3 of diagnosis	3.4 ± 0.7	2.31 ± 0.72	0.056
Day 7 of diagnosis	3.18 ± 0.54	2.34 ± 0.79	0.16

The statistically significant outcomes with P values <0.05 have been highlighted in bold

IVH intraventricular hemorrhage, *ACA* anterior cerebral artery, *PSV* peak systolic velocity, *EDV* end diastolic velocity, *RI* resistive index, *PI* pulsatility index, *NEC* necrotising enterocolitis, *SMA* superior mesenteric artery, *AKI* acute kidney injury, *RA* renal artery

the brain, gut and kidneys using color Doppler ultrasonography of the major supplying arteries to these organs, namely, anterior cerebral, superior mesenteric and renal arteries in LONS. The same may be useful in real-time assessment of organ perfusion, thereby individualising management in sepsis physiology.

To evaluate the changes in blood flow velocities and vascular resistance, the case-cohort was studied against a control group with matching baseline characteristics. A delay in cord clamping has been proven to have a stabilising effect on neonatal hemodynamics, especially during the changes of transitional circulation at birth [16, 17]. It was observed that neonates in both groups received it comparably. Majority (95%) of the neonates did not require any vasoactive medications during the sepsis episode.

The PSV in ACA was higher in neonates with sepsis along with RI and PI values. Higher PSV may be associated with cerebral vascular changes secondary to compensatory mechanisms for maintaining vital organ perfusion in neonatal sepsis. In a previous study by Yengkhom et al. [8], LONS was associated with high RI in ACA, attributed to decreased cerebral blood flow. Values of EDV were also found to be lower in neonates with sepsis with statistical significance in the present study. Similar studies in the past have also shown altered cerebrovascular reactivity as well as intact cerebral autoregulation in neonatal sepsis [18–21]. Almost half of the babies with LONS had IVH. This finding along with the data from previous studies, may point towards a predisposition to cerebral hemorrhage as well as ischemic injury [22].

While there was no statistical significance, values of PSV and PI measured in SMA were found to be slightly higher in neonates with sepsis. This may increase the risk of NEC, owing to impaired splanchnic circulation and was supported by the observation that half of the babies developed NEC in the LONS group as against 15% in the controls. In previous studies, the SMA blood flow velocities have been correlated with early feeding tolerance and NEC [23, 24]. Thus, monitoring splanchnic circulation in real-time may aid in assessing the risk of feed intolerance and NEC in LONS.

All the blood flow markers for renal circulation were altered in neonates with sepsis. The EDV values were lower, while PSV, RI, and PI were elevated in LONS. The above findings may indicate impaired renal perfusion and renal dysfunction predisposing to AKI [25]. Thus, monitoring the urine output in neonates with sepsis is warranted.

Strikingly, the abnormal blood flow parameters in all arteries persisted until day 7 of LONS, thus stressing the importance of continuous monitoring of vital parameters indicative of organ perfusion for a longer duration. A watchful assessment for signs of encephalopathy, NEC, kidney injury, as well as septic shock, and multi-organ dysfunction in these neonates is warranted.

Although an overall analysis suggested that Doppler velocimetry parameters are altered in LONS, when subgroup analysis for the same was performed for neonates with and without clinical outcomes, significant inference could not be drawn. Similarly, the subgroup analysis of the Doppler velocimetry parameters in the neonates with LONS could not suggest a significant inference. This may have been due to the limited number of neonates in the assigned groups.

The strengths of our study include serial evaluation of blood flow over a period of time, thus, allowing a detailed assessment of systemic perfusion along with its clinical correlation. To our knowledge, this is the first study to evaluate multi-organ perfusion simultaneously and comprehensively with most of the markers of Doppler ultrasonography included and assessed for serial fluctuations.

The limitation of this study was small sample size. Although, the Doppler parameters were serially evaluated, a detailed correlation with cardiac function, feeding status and clinical outcomes in real-time could not be done. Additionally, less number of extremely preterm neonates were a part of the study. While Doppler parameters may aid in assessment of organ perfusion, correlation of the same in light of more specific strategies for determination of blood flow such as near infra-red spectroscopy and organ specific biomarkers was not done and thus, remains a point of interest for future studies.

Conclusion

Our study shows alterations in cerebral, splanchnic and renal perfusion by means of abnormal blood flow velocimetry and vascular resistance. This may prove to be a more accurate surrogate marker of alterations in systemic circulation in LONS as compared to the monitoring of vital parameters alone. Additionally, it can anticipate and guide appropriate management of adverse outcomes such as neonatal encephalopathy, intra-cerebral bleeds, NEC and AKI. However, a larger multicentric study, with a higher proportion of extremely preterm neonates, would be useful to understand this hemodynamic association as well as its correlation with associated complications and neonatal outcomes. Future research encompassing evaluation of Doppler flow velocities as a marker for culture-positive sepsis and its diagnostic accuracy, and detailed assessment of hypoperfusion-reperfusion injury mediated systemic complications should be studied for a comprehensive understanding of hemodynamic disturbances in late-onset sepsis.

Declarations

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