ORIGINAL RESEARCH

Automated Cell Counter-derived Neutrophil Cell Population Data by VCS Technology as a Marker of Early-onset Neonatal Sepsis

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Abstract

Aim: Early-onset neonatal sepsis (EONS) occurring within the first 72 hours after birth is a common, life-threatening disease in neonatal intensive care units (NICUs). The limited accuracy of diagnostic tools makes the diagnosis of EONS difficult, and the quest for new markers remains open. Automated hematology analyzer-derived neutrophil cell population data (N-CPD) have been identified as a potential marker of neonatal sepsis, but their role for EONS has not been elucidated yet. Our aim is to explore the role of automated hematology analyzer-derived N-CPD as a marker of EONS.

Methods: We prospectively evaluated a cohort of 289 neonates admitted to the NICU with clinical signs of sepsis, and checked if N-CPD from the Beckman Coulter UniCel DxH 800 device could help identify those who would develop culture-proven EONS. Clinical characteristics, sepsis markers, blood culture results, and N-CPD were recorded. The diagnostic accuracy of N-CPD was tested using receiver-operator curves (ROCs).

Results: Receiver-operator curves of the standard deviation of neutrophil volume (SD-V) showed moderate accuracy in identifying EONS (AUC 0.74), with a high negative predictive value (NPV 98.6%) for cut-off values >21.76 arbitrary units. Accuracy was higher with VCS at 12–48 hours of life (AUC 0.8). Standard deviation of neutrophil volume accuracy was independent from gestational age (GA), birth weight, and timing of test execution (OR 1.14, $p = 0.002$; AUC 0.71).

Conclusion and significance: Our study confirms the role of N-VCS in the diagnostic workup for EONS. High NPV values may be useful as they suggest a role as an adjunctive marker useful for ruling-out EONS and support early empirical antibiotic withdrawal.

Keywords: Automated cell counter, Case-control study, Cell population data, Diagnosis, Early-onset neonatal sepsis, Markers, Neutrophils.

Newborn (2022): 10.5005/jp-journals-11002-0030

Introduction

Early-onset neonatal sepsis is defined as a positive blood or cerebrospinal fluid bacterial culture at less than 72 hours of age and is the most common life-threatening, vertically-transmitted bacterial infection in NICUs.[1] The overall estimated incidence of EONS is 0.98 per 1,000 live births, with 10.9% mortality. Both the incidence and mortality are inversely proportional to the infants' GA and birth weight.[2,3] Given the ambiguity in the clinical picture and the risk of rapid progression, neonatal sepsis needs prompt investigation and treatment.

Currently, the diagnosis of neonatal sepsis requires a positive blood culture. However, its power is undermined by difficulties in obtaining adequate blood volumes, levels of bacteriemia below a certain detectable threshold, and exposure to antenatal antibiotics that may limit bacterial growth. Hence, there is a need for adjunctive diagnostic tests that are not based on blood culture. Some of these tests include the measurement of the total and differential white blood cell (WBC) counts, the absolute neutrophil counts (ANCs), and the ratio of immature-to-total (I/T) neutrophils.[4,5] However, these tests are limited by low sensitivity and empiric antibiotic therapy still remains necessary for neonates with clinically suspected EONS.[6]

Leukocyte quantitative parameters such as the cell population data (CPD) can be measured using automated hematology analyzers.[7] The Beckman Coulter UniCel DxH 800 is an example; it obtains CPD such as cell volume, conductivity, and scatter (VCS). The VCS technology examines the biophysical properties of 8,000 leukocytes in each specimen, refining the output for increased accuracy. A link between acute bacterial infection and morphologic changes of reactive neutrophils, detected by the VCS technology, has been demonstrated in adults.[8] Preliminary studies show similar changes in neonatal sepsis, but most studies have included both EONS and late-onset neonatal sepsis (LONS).[9–14] In this study, we focused on EONS and evaluated neutrophil VCS parameters to assess the diagnostic accuracy of these parameters in these infants.

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How to cite this article: Morotti F, Candela GF, Martellosio G, *et al*. Automated Cell Counter-derived Neutrophil Cell Population Data by VCS Technology as a Marker of Early-onset Neonatal Sepsis. Newborn 2022;1(2):209–214.

Source of support: Nil

Conflict of interest: None

Methods

Study Population and Sepsis Screening

The recruitment was conducted prospectively from January 2018 to June 2019 at the NICU of the Children's Hospital of Brescia, Italy. The study received approval from the Ethics Committee of ASST-Spedali Civili of Brescia, Italy. Eligibility criteria included inborn neonates admitted to NICU, with clinical suspicion of sepsis within the first 72 hours after birth. We included infants in who a blood culture had been obtained at the time of NICU admission, and they had at least one complete blood count (CBC), including the ANC and VCS parameters, manual I/T ratio, and the C-reactive protein levels measured within the first 72 hours after birth; and (c) The availability of parental written informed consent. Neonates with congenital or chromosomal abnormalities, isoimmunization, or maternal preeclampsia were excluded.

Recruited neonates were observed for 72 hours after birth. There were two groups: (a) Those with blood culture-proven EONS; and (b) Controls with negative blood culture and negative sepsis screening defined as C-reactive protein ≤10 mg/L, WBC counts ≥5000/mm^3, ANC >1000/mm^3, and I/T ratios ≤0.2. Neonates with mixed results such as a positive sepsis screening but negative blood cultures were considered to be potential confounders, and therefore, excluded from the analysis.

Neutrophil Cell Population Data by VCS Analysis

We used the UniCel DxH 800 hematology analyzer (Beckman Coulter, Miami, Florida, USA) to perform CBCs and CPD by VCS analysis. The VCS technology uses three independent energy sources to evaluate the biophysical properties of leukocytes, namely, direct current impedance to measure cell volume (V); radio frequency opacity that is related to intracellular features such as the cytoplasmic/nuclear ratio, to characterize conductivity (C); and a laser beam to measure multiple angles of light scatter (S) for cytoplasmic granularity and nuclear structure.[15] Four angles of light scatter were available, including the median angle light scatter (MALS), the upper median—(UMALS), lower median—(LMALS), low angle—(LALS), and the axial light loss (ALL). With these parameters, the VCS analysis provides information on cell volume (V), cell conductivity (C), and scatter (S), which are reported as mean (MN) and standard deviation (SD).

Statistical Analysis

Statistica (StatSoft Inc., Tusla, Oklahoma, USA) and MedCalc (MedCalc Software, Mariakerke, Belgium) software were used. The characteristics of the two groups of neonates were reported using descriptive statistics. Continuous variables were presented as the median and interquartile range (IQR) and compared by the Mann–Whitney *U* test. Categorical variables were presented as absolute numbers and percentages and compared using Fisher's exact test. Receiver operating characteristic (ROC) curves were analyzed to estimate the accuracy of VCS parameters in predicting EONS. The overall test performance was expressed as the area under the ROC curve (AUC) with a 95% confidence interval (CI). Youden index and its associated cut-off value, for which both sensitivity and specificity are maximized, were determined.[16] A prevalence value of 3.8%, corresponding to the local area-based average value of proven EO sepsis, was used to calculate the positive and negative predictive values. Finally, multivariate logistic regression analysis was done to evaluate the association between VCS parameters and the occurrence of EONS, independently from possible confounders. All statistical tests were considered significant for *p*-values <0.05.

Results

Demographic Characteristics

During the study period, 544 neonates were admitted to NICU. We found 289 to be eligible for the study, and 262 were excluded. Fifteen were excluded because of consent denial, 63 were out-born, 24 had congenital or chromosomal abnormalities, 13 had isoimmunization, 10 were born to mothers with pregnancy-induced hypertension, 7 because of missing data, and 137 did not receive sepsis workup.

Among the 289 enrolled infants, 31 (10.7%) with positive blood cultures were included in the sepsis group, and 198 (68.5%) with negative sepsis screening and negative blood culture and were labelled as controls. To minimize confounders, 58 neonates (20.4%) with positive sepsis screening but negative blood culture were excluded from the data analysis. One more neonate (0.4%) was excluded from data analysis because of overt blood culture contamination.

Clinical Characteristics

Clinical and laboratory characteristics of neonates in the sepsis or control group are summarized in Table 1. Cesarean section (CS) was the mode of delivery in 75% of the control group and 55% of the sepsis group ($p = 0.02$). Neonates in the sepsis group had significantly lower GA and birth weight compared to controls. The timing of sepsis screening was similar between the two groups. Among laboratory tests, values of red blood cells, I/T ratio, and C-reactive protein were significantly different, whereas WBC, ANC, and platelets values were not.

Blood culture results are shown in Table 2. Gram-negative bacteria were detected in 25 cases (80.6%) of positive blood cultures. The gram-positive bacteria included group B Streptococci (five cases, 16%), coagulase-negative *Streptococcus* (five cases, 16%), and *Enterococcus faecalis* (five cases, 16%). Gram-negative bacteria were present in six cases (19.4%), with *E. coli* being the most frequent (four cases, 12.9%). No fungi were detected.

Neutrophil—CPD Data

Patients received a total of 315 CBC with VCS parameters analysis. The cumulative distribution of CBC tests over time is shown in Figure 1. We compared the neutrophil VCS parameters in the sepsis and control groups. Among the mean channel values of VCS, all the parameters were significantly different, except for the MN-LALS. In addition, all parameters of VCS standard deviations resulted significantly different between the two groups, except for conductivity. VCS parameters with statistically significant differences (p <0.01) between the two groups are summarized in Table 3.

Receiver Operating Characteristic (ROC) Curves

Results of the ROC curve analysis of VCS parameters are summarized in Table 4. According to the Swets classification,[17] the accuracy of SD-V results was moderate in predicting EO sepsis (AUC of 0.74) with a sensitivity of 76.9% and an NPV of 98.6% for a cut-off value of >21.76 au (arbitrary unit). All the other VCS parameters were less accurate (AUC ≤0.7). Similarly, the accuracy of WBC and ANC values was low, while the accuracy of the I/T ratio resulted in moderate (AUC of 0.71).

Standard deviation of neutrophil volume ROC curve was analyzed at different timing of sepsis screening, as reported in Table 5. The cut-off value of SD-V, calculated at different cumulative times of sepsis screening, remained the same over the period of 72 hours after birth. Furthermore, the AUC values

Table 1: Newborn characteristics

	Sepsis (n = 31)	*Controls (n = 198)*	*p value*
Male/female	19/12	116/82	0.776
Birth cesarean/vaginal	17/14	148/50	0.021
Gestational age, weeks	30 (26–34)	33 (31–35)	0.004
Birth weight, gm	1,465 (860–2,000)	1,855 (1,461–2,423)	0.008
Sepsis work-up time after birth, hours	22 (11–34)	19 (2–31)	0.222
WBC, *n*/μL	11,058 (8,452–16,686)	11,017 (7,985–15,032)	0.825
RBC, $N \times 10^3$/μL	4,066 (3,463–4,421)	4,405 (3,931–4,869)	0.004
PLT, $n \times 10^3$/μL	205 (163–269)	219 (177–263)	0.666
ANC, *n*/μL	6,152 (3,769–10,662)	5,825 (3,535–9,190)	0.453
I/T ratio	0.08 (0.06–0.18)	0.04 (0.03–0.07)	<0.001
CRP mg/L	3.7 (0.0–10.4)	0.0 (/)	<0.001

Values are reported as median, (interquartile range). ANC, absolute neutrophil count; CRP, C-reactive protein; I/T, immature to mature neutrophils ratio; RBC, red blood cells; WBC, white blood cells

Table 2: Blood culture results; number and (percentage)

	N (%)
Gram-positive organisms	25 (80.6)
Group B streptococci	5 (16.1)
Coagulase-negative staphylococci	5 (16.1)
Enterococcus faecalis	5 (16.1)
Gram-positive cocci (other)	4 (12.9)
Staphylococcus lugdunensis	2 (6.5)
Corynebacterium sp.	2 (6.5)
Turicella otitidis	1 (3.2)
Lactobacillus jensenii	1 (3.2)
Gram-negative organisms	6 (19.4)
Escherichia coli	4 (12.9)
Haemophilus influenzae	2 (6.5)

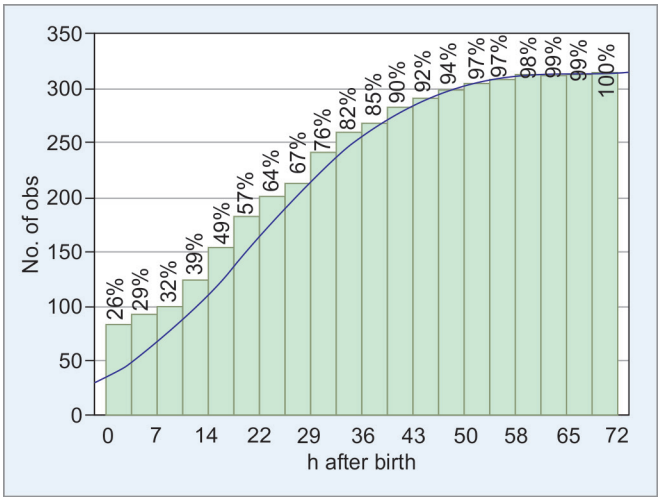


Fig. 1: Cumulative distribution of CPD determination over time

remained greater than 0.70, with the best performance for tests taken between 12 and 48 hours, with an AUC of 0.8 (Fig. 2). Although the positive predictive value was 24.2% at best, the negative predictive value was stably higher than 95%. By logistic regression, SD-V was significantly associated with EO sepsis independently from GA, birth weight, and timing of test execution [OR 1.14 (95% CI 1.05–1.24), $p = 0.002$; AUC 0.71].

Discussion

In EONS, the relatively-modest diagnostic accuracy of CRP and the long turn-around time of blood cultures makes prompt diagnosis a difficult task.[18] To overcome such limitations, several EONS management strategies have been evaluated, including clinical algorithms[19,20] and logistic regression models (Kaiser Permanente, California, USA). Despite some limitations, these efforts have helped reduce unnecessary examinations and empirical antibiotic therapy in at-risk late preterm or term newborn infants. Unfortunately, these methods have been designed primarily for near-term infants who are usually healthier and more mature than the premature infants admitted to the NICUs.

We are still looking for novel, accurate diagnostic methods to screen less mature preterm infants who are at risk of EONS. Several markers have been investigated so far, such as procalcitonin (PC), interleukin 6 (IL-6), presepsin (PS), or CD64, but a reliable, cost-effective test has not been found.[18] Along with CRP, CBC and peripheral blood smears are routinely used as tests for neonatal sepsis workup, with I/T ratio being a useful additional sepsis marker (sensitivity 90%, NPV 98–99%, PPV 25% for I/T >0.2).[18] Nevertheless, the manual examination is usually conducted with a time-consuming, direct observation of only 100–200 cells, is operator-dependent, and is subject to sampling variations. The optimal diagnostic assay is still far from being identified.

Modern hematology analyzers use multiple physical parameters to evaluate and classify the cells of a specimen. The Coulter UniCel DXH800 employs the VCS technology to examine CPD. When applied to leukocytes, the VCS parameters can be considered as a fully-automatized, highly objective, and reproducible equivalent of morphological parameters. The initial data are exciting for sepsis evaluation.[8] To our knowledge, six studies assessing N-CPD performance in neonatal sepsis are available so far.[9–14] Raimondi et al.[9] found MN-V and neutrophils SD-V to be useful in excluding bloodstream infection in LONS, with AUC, sensitivity, specificity and NPV of 0.92, 95.0, 88.0, 98.9% and 0.75, 80.0, 52.0, 92.8%, respectively. The other authors described N-CPD performance in mixed early and late-onset sepsis cohorts. With some differences, all found MN-V interesting, with a mean cut-off >155.9 au (range 151–157.1 au), and

Table 3: Neutrophil VCS parameters

VCS parameters (AU)	*Total (315)*	*Sepsis (n = 31)*	*Controls (n = 198)*	*p value*
MN-V	141 (136–148)	146 (137–158)	141 (136–147)	<0.001
SD-V	21.9 (19.6–23.2)	23.86 (21.8–26.4)	20.92 (19.5–22.6)	<0.001
MN-C	139 (136–142)	137 (133–142)	139 (137–142)	0.006
MN-MALS	130 (127–135)	127 (122–131)	131 (128–135)	<0.001
MN-UMALS	136.9 (133–142)	133 (129–140)	138 (134–142)	0.001
SD-UMALS	15.4 (12.9–15.9)	15.58 (13.7–20)	14.01 (12.9–15.6)	0.002
MN-LMALS	119 (115–125)	116 (112–120)	120 (116–125)	<0.001
SD-SAL2/ALL	16.5 (14.7–17.3)	17.00 (15.9–21.5)	15.72 (14.5–17)	<0.001

Measurement units were arbitrary units (AU). Values are reported as median, (interquartile range). ALL, axial light loss; C, cell conductivity; LMALS, lower median angle light scatter; MALS, median angle light scatter; MN, mean channel; SD, standard deviation; UMALS, upper median angle light scatter; V, cell volume

Table 4: ROC curve analysis for selected VCS parameters and white blood count (WBC), absolute neutrophils count (ANC), immature to mature neutrophil ratio (I/T)

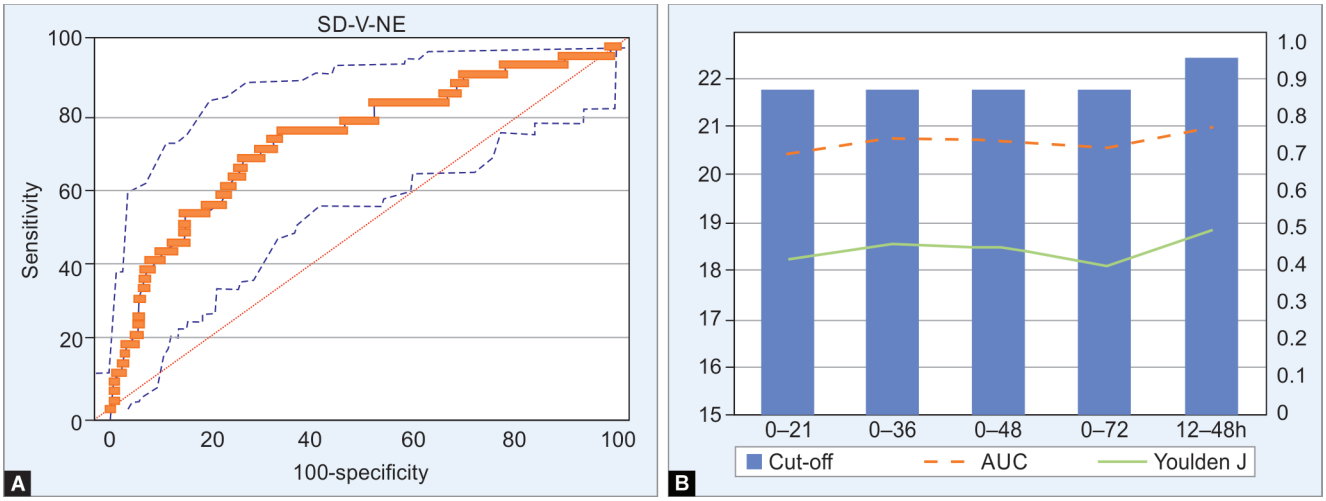
VCS parameters	*Cut-off (AU)*	*AUC*	*Sensitivity (95% CI)*	*Specificity (95% CI)*	*PLR (95% CI)*	*NLR (95% CI)*	*PPV (95% CI)*	*NPV (95% CI)*
MN-V	>149	0.67	48.7 (32.4–65.2)	85.9 (81.2–89.8)	3.5 (2.2–5.3)	0.6 (0.4–0.8)	12 (4.4–24.5)	97.7 (95.1–99.1)
SD-V	>21.76	0.74	76.9 (60.7–88.9)	65.9 (60.0–71.5)	2.3 (1.8–2.9)	0.4 (0.2–0.6)	24.2 (17.0–32.7)	95.3 (91.2–97.8)
MN-C	≤134	0.63	38.5 (23.4–55.4)	88.4 (84.0–91.9)	3.3 (2.0–5.5)	0.7 (0.5–0.9)	11.6 (3.6–25.7)	97.3 (94.7–98.9)
MN-MALS	≤126	0.68	48.72 (32.4–65.2)	81.88 (76.8–86.2)	2.69 (1.8–4.0)	0.63 (0.5–0.9)	9.6 (3.5–19.9)	91.9 (87.7–95.0)
MN-UMALS	≤133	0.66	51.28 (34.8–67.6)	78.26 (72.9–83.0)	2.36 (1.6–3.4)	0.62 (0.4–0.9)	8.5 (3.2–17.5)	97.6 (94.8–99.1)
SD-UMALS	>14.44	0.65	74.36 (57.9–87.0)	51.09 (45.0–57.1)	1.52 (1.2–1.9)	0.50 (0.3–0.9)	5.7 (2.6–10.5)	98.1 (94.5–99.6)
MN-LMALS	≤118	0.67	69.23 (52.4–83.0)	60.87 (54.8– 66.7)	1.77 (1.4–2.3)	0.51 (0.3–0.8)	6.5 (2.9–12.3)	98 (88.6–96.5)
SD-SAL2	>16.46	0.69	66.67 (49.8–80.9)	64.86 (58.9–70.5)	1.90 (1.4–2.5)	0.51 (0.3–0.8)	7 (3.1–13.3)	98 (94.9–99.5)
WBC (cell/µL)	>21,220	0.5	18.4 (7.7–34.3)	94.2 (90.8–96.7)	3.18 (1.4–7.2)	0.87 (0.7–1)	11.2 (1.6–33.4)	96.7 (94–98.4)
ANC (cell/µL)	>15,586	0.53	18.4 (7.7–34.3)	95.6 (92.5–97.7)	4.24 (1.8–10)	0.85 (0.7–1)	14.3 (2.1–41.3)	96.7 (94–98.4)
I/T (ratio)*	>0.05	0.71	75.8 (56.5–89.7)	62.6 (54.8–69.2)	2 (1.5–2.5)	0.39 (0.2–0.7)	7.3 (2.8–15.1)	98.5 (94.6–99.8)

*I/T values were provided only for 29 cases, 185 controls. NLR, neutrophil to lymphocyte ratio; NPV, negative predictive ratio; PLR, platelet to neutrophil ratio; PPV, positive predictive ratio

Table 5: SD-V ROC curve analysis for different timings of sampling

Timing	*Number Case/ctrl*	*Cut-off (AU)*	*AUC*	*J*	*Sensitivity (95% CI)*	*Specificity (95% CI)*	*PLR (95% CI)*	*NLR (95% CI)*	*PPV (95% CI)*	*NPV (95% CI)*
0–24 hours	20/176	>21.76	0.72	0.44	75 (50–91.3)	69.32 (61.9–76.0)	2.44 (1.7–3.4)	0.36 (0.2–0.8)	8.8 (3.2–18.6)	98.6 (94.8–99.8)
0–36 hours	32/230	>21.76	0.77	0.48	80.6 (62.5–92.5)	67.4 (60.9–73.5)	2.47 (1.9–3.2)	0.29 (0.1–0.6)	8.9 (3.9–16.8)	98.9 (95.9–99.9)
0–48 hours	35/259	>21.76	0.76	0.47	80 (63.1–91.6)	67.18 (61.1–72.9)	2.44 (1.9–3.1)	0.3 (0.2–0.6)	8.8 (4.1–16.1)	98.8 (96.1–99.8)
0–72 hours	39/276	>21.76	0.74	0.42	76.9 (60.7–88.9)	65.9 (60.0–71.5)	2.3 (1.8–2.9)	0.4 (0.2–0.6)	24.2 (17.0–32.7)	95.3 (91.2–97.8)
12–48 hours	25/164	>22.4§	0.8	0.52	80 (59.3–93.2)	72.53 (65.1–79.2)	2.92 (2.1–4)	0.28 (0.1–0.6)	9.3 (3.2–20.2)	99 (95.6–100)

Measure unit: arbitrary unit (AU). §Value of 21.76 not included in sample. NLR, neutrophil to lymphocyte ratio; NPV, negative predictive ratio; PLR, platelet to neutrophil ratio; PPV, positive predictive ratio



Figs 2A and B: (A) ROC curve, SD-V; (B) SD-V performance at different sample timings

mean AUC of 0.84 (0.8–0.99), sensitivity, specificity, and NPV of 75.9% (35–97%), 85.2% (71.9–96%), 80.5% (65–98%), respectively. Standard deviation of neutrophil volume was also indicated by four studies, with a mean cut-off >29.7 AU (range 21.5–37.4), and mean AUC of 0.76 (0.68–0.9), sensitivity, specificity, and NPV of 70.4% (60–88%), 72.8% (64–78%), 74.7% (48–92%), respectively.[11–14] However, the authors considered both EONS and LONS together: Their results may not be fully applicable to EONS, as during the early neonatal period the neutrophil kinetics is known to show high variability and physiologic changes.[5] Moreover, most authors included infants with both culture-proven and variously defined suspected/clinical sepsis in the "sepsis group". This approach may have diluted the diagnostic accuracy of various kinetic parameters of neutrophils.[9,12,14]

To our knowledge, this is the first reported study specifically evaluating neutrophil CPD for predicting EONS. Our sample includes 31 culture-proven EONS cases, matched with 198 controls. Blood samples were collected as per routine internal protocol or clinical indication. Cases and controls significantly differed for GA, birth weight, and mode of delivery. Nevertheless, none of these data, as well as the timing of blood sampling, had an influence on the N-CPD predictive value, as shown by logistic regression. Among sepsis screening tests, I/T ratio, CRP, and several N-CPD showed a significant difference between the sepsis group and controls. Our NICU can rely on long-time experienced physicians for I/T ratio execution. The close similarity of I/T and SD-V performance at the ROC curve suggests a biological link between the two measurements.

In most previous studies, MN-V and SD-V were identified as best performing parameters. We focused on the best-performance VCS parameter SD-V and MN-V. Standard deviation of neutrophil volume >21.76 au showed an AUC of 0.74, 76.9% sensibility, and 65.9% specificity, with an NPV of 95.6%. The cut-off value, AUC, and Youden index's J remained constant when analyzed for different time slots (Fig. 2). Interestingly, the best performance was obtained with VCS analysis performed at 12–48 hours, with AUC of 0.8 and NPV of 99%, suggesting that the timespan is optimal for N-CPD evaluation.

Compared to existing data, our focused analysis of N-CPD in infants with EONS has shown interesting results. Standard deviation of neutrophil volume was more accurate than previously reported, showing more variability of neutrophils in EONS due to enhanced margination of neutrophils at diverse stages of immaturity. In contrast, the lower AUC values of MN-V for EONS could be explained by the higher number of large, immature neutrophils physiologically present in the circulation in the first hours after birth. These results highlight the biological difference between early and late neonatal sepsis response, which should be taken into account when studying potential neutrophil morphology derived parameters. Our study does have some limitations, particularly in its small population size. Nevertheless, these preliminary data that are specifically oriented to EONS suggest that the results could be reliable for clinical use. Standard deviation of neutrophil volume showed moderate accuracy along with a high negative predictive value, as well as stability over the first 48 hours. Compared to other laboratory tests, N-CPD can be generated automatically with routine CBCs and does not require additional blood specimens, are available within a few minutes, and once established, there are no additional costs. This could very well develop into an important adjunctive marker in the work-up for EONS.

Conclusion

Neutrophil cell population data can be a cost-effective and time-sparing laboratory evaluation that could provide important adjunctive markers for routine EONS workup. The high negative predictive value makes SD-V a useful parameter for ruling-out EONS. The low PPV and specificity can limit its use as an individual test, but it could be very useful in combination with other sepsis workup examinations to improve EONS diagnosis and refine antimicrobial stewardship programs.

Acknowledgments

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript. The authors have no relevant financial or non-financial interests to disclose. Ethics approval was granted by the Ethics Committee of the ASST-Spedali Civili di Brescia Hospital.

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