Circulating Neutrophils in Septic Preterm Neonates: Comparison of Two Reference Ranges

William D. Engle, MD*; Charles R. Rosenfeld, MD*; Anna Mouzinho, MD; Richard C. Risser‡; Fiker Zeray, RN*; and Pablo J. Sanchez, MD*

ABSTRACT. *Objective.* **To study the effect of sepsis on circulating neutrophils in very low birth weight neonates and to assess the usefulness of recently revised reference ranges for circulating neutrophils in the diagnosis of sepsis in this population by comparison with previously reported reference ranges.**

Methods. **Neutrophil parameters (absolute total neutrophils, absolute total immature neutrophils, and the immature:total neutrophil proportion) were analyzed retrospectively in 202 sepsis episodes in 192 neonates (birth weight = 1055 ± 246 g, X ± SD; estimated gestational age = 29 ± 2 weeks) between birth and 30 days of age. The percentage of values lying outside the reference ranges reported recently by Mouzinho et al[32] and previously by Manroe et al[24] were compared. To more accurately assess possible differences in specificity between the two reference ranges, neonates with early-onset group B streptococcal infection (n = 19) were compared with a matched control group (n = 51) using conditional logistic regression.**

Results. **Greater sensitivity was observed using the previous reference ranges of Manroe et al[24] over the entire study period (0 to 720 hours) both for the initial and the second complete blood count (CBC). The previous reference ranges also were more sensitive than the revised ranges for the initial CBC at 0 to 72 and at 73 to 720 hours and for infections attributable to coagulase-negative staphylococci. However, specificity in neonates without group B streptococcal infection was significantly greater with the revised reference ranges compared with those of Manroe et al[24] (initial CBC, 73% vs 45%; serial CBCs, 59% vs 10%).**

Conclusion. **The observed differences in sensitivities may be of limited clinical significance because very low birth weight infants often are begun on antibiotic therapy regardless of laboratory values. However, the striking differences in specificity using the revised reference ranges suggest that these ranges may be clinically useful in determining length of antimicrobial therapy in infants in whom cultures remain sterile.** *Pediatrics* **1997;99(3). URL: http://www.pediatrics.org/cgi/content/full/99/3/e10;** ***sepsis, very low birth weight neonate, neutrophils.***

From the Departments of *Pediatrics and ‡Academic Computing, University of Texas Southwestern Medical Center, Dallas, TX 75235–9063.

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Reprint requests to (W.D.E.) University of Texas Southwestern Medical Center at Dallas, Department of Pediatrics, 5323 Harry Hines Blvd, Dallas, TX 75235–9063.

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ABBREVIATIONS. VLBW, very low birth weight; CBC, complete blood count; BW, birth weight; GBS, group B streptococcal (infection); ATI, absolute total immature neutrophil count; I:T, immature neutrophil:total neutrophil proportion; ATN, absolute total neutrophil count; PPV, positive predictive value; NPV, negative predictive value.

The timely diagnosis of sepsis in the very low birth weight (VLBW; ≤1500 g) neonate is critical because the illness can be rapidly progressive and in some instances fatal.[1,2] Numerous investigators have evaluated the usefulness of various laboratory tests in the diagnosis of systemic infection in this population.[3–6] These include determination of C-reactive protein,[7] erythrocyte sedimentation rate,[8] haptoglobin,[9] orosomucoid,[10] fibronectin,[5] elastase-α-1-proteinase inhibitor complex,[11] C3d,[12] endotoxin,[13] acridine orange cytospin,[14] and nitroblue tetrazolium reduction.[15] Although multiple tests have been used together for this purpose, perhaps the single test of greatest utility in establishing a diagnosis of sepsis has been the complete blood count (CBC) and in particular the various neutrophil parameters.[2,16–23] In studies reported in 1979,[24] we established reference ranges for circulating neutrophils in neonates with postnatal age ≤30 days. Although the gestational age and birth weight (BW) of these neonates ranged from 29 to 44 weeks and from 860 to 5000 g, respectively, these data were obtained between 1974 and 1976, when the survival of preterm, VLBW neonates was substantially less than that observed more recently. In this report and others, neutropenia was associated with neonatal sepsis, especially attributable to group B streptococcus (GBS).[25,26]

Based on subsequent studies from our institution and elsewhere,[27–31] it appeared, however, that many normal VLBW neonates were considered to be neutropenic using the references ranges of Manroe et al.[24] More recently, we reported revised reference ranges for circulating neutrophils in VLBW neonates.[32] Although the reference ranges for absolute total immature neutrophils (ATI) and the immature: total neutrophil proportion (I:T) were unchanged, the reference ranges for absolute total neutrophils (ATN) differed from those previously reported. There was a broader distribution for ATN values during the first 72 hours after birth, primarily reflecting a markedly lower limit throughout this period of time and a shift in the peak upper range from 10 to 12 hours to 18 to 24 hours after birth (see Figure). Because more VLBW neonates would now have

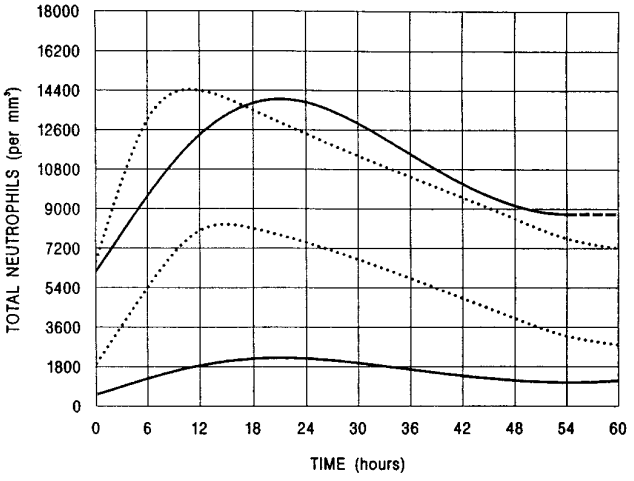

Figure. Reference ranges for total neutrophil values in the first 60 hours after birth. The solid lines depict the boundaries of the revised reference range of Mouzinho et al[32] in infants ≤1500 g. The dotted lines depict the reference range of Manroe et al.[24] For the latter, the minimum value of 1750 total neutrophils/mm^3 is established by 72 hours of age; a stable maximum value of 5400 neutrophils/mm^3 is reached at 120 hours.

ATN values that would fall into the normal range, we hypothesized that the usefulness of peripheral neutrophil values in helping to establish a diagnosis of sepsis in this population might be diminished. To address this concern, we analyzed circulating neutrophil values obtained from 192 VLBW neonates born between 1987 and 1993 with proven sepsis in the first postnatal month. Neutrophil values were examined using the reference ranges of Manroe et al[24] as well as those of Mouzinho et al[32] to assess differences in sensitivity between the two methods. In addition, a subset of the population [19 infants with early-onset (0 to 72 hours) GBS infection] was compared with a matched control group to assess differences in specificity between the reference ranges of Manroe et al[24] and Mouzinho et al.[32]

METHODS

Subjects

The study population consisted of 192 VLBW neonates delivered at Parkland Memorial Hospital between January 1, 1987 and December 31, 1993 who had a positive bacterial or fungal isolate from blood and/or cerebrospinal fluid in the first month of life (Table 1). The 192 study infants had a total of 202 episodes of culture-proven sepsis identified by prospective surveillance and they represented 11% of the 1732 neonates with BW ≤1500 g admitted to the neonatal intensive care unit during the study period. Furthermore, they represented all infants in this BW group with proven sepsis and at least one CBC obtained at the time of the evaluation for sepsis. Four infants with sepsis were excluded because CBC data was unavailable. A subset of this population, ie, 19 neonates with early-onset GBS infection, is described in Table 2. One infant with early onset GBS was excluded because CBC data was unavailable. None of the mothers of these 19 infants received intrapartum antibiotics. Using a preexisting database for all VLBW neonates admitted to the intensive care nursery, we subsequently identified a control group consisting of 51 neonates without early-onset infection who were matched with those with GBS disease using the date of birth, sex, race, gestational age (within 3 weeks) and BW (within 15%). All matched neonates were born within 10 months of the index case who had GBS disease; for 11/19 index cases (58%), control neonates were born within 30 days (Table 2). Between 2 and 4 neonates were matched for each index cases with CBCs obtained at similar postnatal ages in the first 72 hours of life.

TABLE 1. Characterization of Study Population

Number of infants	192
Birth weight (g) range*	1055 ± 246 (570–1500)
Gestational age (wk) range*	28.7 ± 2.2 (24–34)
Sex	
Males	109 (57)†
Females	83 (43)
Race	
African-American	103 (54)
Latin-American	44 (23)
White	40 (21)
Asian	5 (2)
Clinical	
Maternal PIH‡	27 (14)
5-min Apgar score ≤5	43 (23)
Respiratory distress syndrome	114 (59)
Mechanical ventilation	164 (85)
Apnea of prematurity	155 (81)

* Values are presented as means ± SD.
† Numbers in parentheses are percentages (except for range).
‡ Pregnancy-induced hypertension.

TABLE 2. Neonates With Early-onset Group B Streptococcal (GBS) Sepsis and Their Matched Controls

	Early-onset GBS	Matched Controls
Number of infants	19	51
Birth weight (g)*	1019 ± 267	1057 ± 270
Gestational age (wk)*	28.5 ± 2.5	28.6 ± 2.6
Sex		
Males	5 (26)†	14 (27)
Females	14 (74)	37 (73)
Race		
African-American	14 (74)	39 (76)
Latin-American	2 (10.5)	6 (12)
White	2 (10.5)	6 (12)
Asian	1 (5)	0 (0)
Clinical		
Maternal PIH‡	2 (11)	11 (22)
5-min Apgar score ≤5	8 (42)	10 (20)
Respiratory distress syndrome	9 (47)	35 (69)
Mechanical ventilation	14 (74)	36 (71)
Apnea of prematurity	10 (53)	37 (73)

* Values are presented as means ± SD.
† Numbers in parentheses are percentages.
‡ Pregnancy-induced hypertension.

Laboratory Methods

CBCs, using a sample obtained by venipuncture, heel stick, or from an umbilical catheter, were determined in all neonates at the time of evaluation for sepsis and were analyzed retrospectively. Sequential values were available for analysis 12 to 24 hours after the initial CBC in 147 neonates (77%). Neutrophil indices were calculated as previously described by Manroe et al.[24] Peripheral blood nucleated cell counts were performed with a Sysmex TM NE 8000 (Toa Medical Electronics Co, Ltd, Kobe, Japan) and a manual 100-cell differential cell count was performed on Wright-stained blood films for white blood cell counts <30 000/mm^3 and on 200 cells if the white blood cell count exceeded 30 000/mm^3. The ATN count was determined from the sum of mature and immature neutrophils. The ATI count included bands and other granulocyte precursors. The I:T proportion was calculated as ATI/ATN. All neutrophil values were determined by the routine hematology lab, the reliability of which has been reported previously.[24,27]

Sepsis was defined as a positive blood and/or cerebrospinal fluid culture in a neonate who was evaluated either shortly after birth or within 30 days after birth attributable to an alteration in clinical course in which infection was considered in the differential diagnosis. Instances in which sepsis was strongly suspected but cultures remained sterile were not included. In the case of coagulase-negative staphylococci, two of two positive blood cultures were required for a diagnosis of sepsis, otherwise the isolate

was considered to be a contaminant and not included in the analysis. Of the 215 causative organisms, 173 (80%) were Gram-positive, 34 (16%) were Gram-negative, and 8 (4%) were Candida species. Of the Gram-positive isolates, 82 (47%) were coagulase-negative staphylococci and 26 (15%) were GBS. In 9 sepsis episodes, more than one organism was isolated.

Statistical Analysis

Sepsis episodes were grouped into those occurring in the first 72 hours after birth (early-onset) and those occurring between 73 and 720 hours (late-onset). ATN values were assessed using the new reference ranges for VLBW neonates[32] and those previously reported by Manroe et al.[24] Differences in the ability of the reference ranges to detect infants with sepsis on the basis of neutrophil values outside the limits were statistically compared by McNemar's test.[33] Data also were analyzed in an organism-specific manner for neonates with sepsis attributable to coagulase-negative staphylococci, Gram-negative organisms, or GBS. Conditional logistic regression was used to compare the early-onset GBS cases and matched controls based on the quantitative neutrophil indices, the characterization of neutrophil indices as normal or abnormal considering both the revised reference ranges and the Manroe et al reference ranges,[24] and the presence of one or more neutrophil index abnormalities considering both set of reference ranges. Sensitivity was defined as the percentage of infants with culture-proven sepsis who had an abnormal neutrophil index value. Specificity was defined as the percentage of matched controls with normal neutrophil index values. Sensitivity and specificity based on the two sets of reference ranges were compared using McNemar's test considering the cases and matched controls, respectively. Positive predictive value (PPV) and negative predictive value (NPV) also are reported for the reference ranges. It should be noted that reporting PPV and NPV directly from a case-control study can be inappropriate because the observed prevalence is an artificial construct of the study design, and PPV and NPV are dependent on prevalence.[34] However, more readily interpretable estimates of PPV (number of infants with sepsis and abnormal neutrophil index value ÷ total number of infants with abnormal neutrophil index value) and NPV (number of control infants with normal neutrophil index values ÷ total number of infants with normal neutrophil index values) can be obtained based on an assumed but more realistic level of prevalence; the PPV and NPV we report are based on an assumed prevalence of 1% attributable to our recent history of 19 cases of early-onset GBS among 1732 VLBW admissions to the nursery.

The nature of this study did not require informed consent as per our Institutional Review Board. Our Institutional Review Board does not require formal approval of a study protocol that involves only retrospective analysis of clinical information and in which no identification of individual patients is made. The prospective surveillance of positive cultures is routine infection control policy in our institution.

RESULTS

Analysis of Sensitivity

There were 202 CBCs obtained at the time of initial evaluation for sepsis from the 192 study infants (10 study infants had more than one sepsis episode during the first 30 days of life). Thirty-two of these were obtained from 0 to 72 hours, while 170 were obtained between 73 and 720 hours. Follow-up CBCs, generally obtained 12 to 24 hours after the initial evaluation, were available in 27 episodes (84%) occurring from 0 to 72 hours and 120 episodes (71%) occurring from 73 to 720 hours. The proportion of neutrophil values outside the reference ranges of Manroe et al[24] and Mouzinho et al[32] is presented in Table 3. In episodes of early-onset sepsis, neutropenia was by far the predominant abnormality of ATN values irrespective of the reference range used. In contrast, neutrophilia was significantly more common in CBCs obtained from 73 to 720 hours after birth ($P <$ .001 for each reference range).

When data from the entire neonatal period (0 to 720 hours) were analyzed, ATN values from both the first and second CBC were more likely to have at least one abnormality using the reference ranges of Manroe et al[24] (81% and 89%, respectively) than with

TABLE 3. Comparison of the Sensitivity of the Revised Reference Range[32] and That of Manroe et al[24] for Neutrophil Values in VLBW Neonates With Proven Sepsis

	ATI†	I:T†	Manroe et al			Mouzinho et al		
			↓ ATN	↑ ATN	Any‡	↓ ATN	↑ ATN	Any‡
All organisms								
0–72 h (32 cases)								
1st CBC (32 counts)	9	50	56	3	75§	13	3	56
2nd CBC (27 counts)	22	67	74	4	96	22	4	78
73–720 h (170 cases)								
1st CBC (170 counts)	43	37	5	64	82‖	5	58	77
2nd CBC (120 counts)	49	41	7	68	88	4	65	85
GBS								
0–72 h (19 cases)								
1st CBC (19 counts)	5	58	58	0	74	16	0	58
2nd CBC (16 counts)	19	81	69	0	94	25	0	88
Coagulase-negative staphylococcus								
73–720 h (78 cases)								
1st CBC (78 counts)	41	30	4	69	85¶	4	60	76
2nd CBC (52 counts)	48	27	6	79	90	4	77	87
Gram-negative bacilli								
73–720 h (26 cases)								
1st CBC (26 counts)	42	50	19	46	89	15	46	89
2nd CBC (21 counts)	48	71	14	43	95	14	43	95

Values are the percent of counts outside the reference range.
† The reference ranges for ATI and I:T values were the same using data of Manroe et al[24] and that of Mouzinho et al.[32]
‡ This indicates that at least one abnormal value was present from ATN, ATI and I:T values.
§ P = .031, Manroe reference range vs Mouzinho reference range.
‖ P = .002, Manroe reference range vs Mouzinho reference range.
¶ P = .016, Manroe reference range vs Mouzinho reference range.

that of Mouzinho et al[32] (73% and 84%, respectively). These differences were significant by McNemar's test ($P < .001$ for initial neutrophil values and $P = .008$ for the second CBC). A similar difference in sensitivity for any abnormality also was noted between the new and old reference ranges for the initial CBC both at 0 to 72 hours ($P = .031$) and at 73 to 720 hours ($P = .002$) (Table 3). However, there were no significant differences between reference ranges for neutrophilic parameters in the second CBC, or when any neutrophil abnormalities from both the first and second CBCs were considered for either early-onset or late-onset sepsis episodes.

Among the 19 cases of early-onset GBS infection, no neonate demonstrated neutrophilia with either of the ATN reference ranges, and the occurrence of neutropenia was ~70% lower with the revised compared with the previous reference ranges. Using the reference ranges of Mouzinho et al, at least one neutrophil value was abnormal in 58% and 88% of first and second CBCs, respectively (Table 3). This did not differ significantly from that seen with the values reported by Manroe et al,[24] 74% and 94%, respectively. Among the 16 infants who had both a first and second CBC performed, comparison of earlier and later neutrophil values failed to show differential sensitivity, regardless of which reference ranges were used.

Because only 3 cases of sepsis attributable to coagulase-negative staphylococci occurred in the first 72 hours, data are reported for only those sepsis episodes (n = 78) that occurred from 73 to 720 hours (Table 3). With the ATN reference range of Mouzinho et al,[32] at least one neutrophil value was abnormal in 76% and 87% of first and second CBCs, respectively, as compared with 85% and 90%, respectively, using the range of Manroe et al. The difference in sensitivity was only significant for the first CBC ($P = .016$). There also was no difference in the sensitivities when abnormalities on either the first or second CBC were considered, 84% and 95%, respectively.

Only 3 cases of sepsis attributable to Gram-negative organisms occurred in the first 72 hours; therefore, data are reported only for the 26 late-onset episodes. As seen in Table 3, the percentage of abnormal neutrophil values was identical using either reference range, ie, ≥89%. Furthermore, the sensitivities using both first and second CBCs were 100% with either ATN reference ranges.

Analysis of Specificity

Results of the comparison between neonates with early-onset GBS infection and their matched, uninfected controls permit an assessment of specificity; these data are presented in Table 4. When the first CBC was examined, ATN and ATI values, whether assessed quantitatively (cells/mm^3) or qualitatively (ie, the relative number within vs outside the reference range), did not differ between GBS cases and controls. Although the quantitative difference in the initial I:T proportion between cases and controls was of marginal significance ($P = .06$), there was a highly significant difference ($P = .002$) when the I:T proportion was assessed qualitatively, ie, 58% of the cases were outside the I:T reference range as compared with 14% of the matched controls.

TABLE 4. Comparison of Neutrophil Indices in Neonates With Proven Early-onset Group B Streptococcal Sepsis and Matched, Uninfected Control Neonates

	GBS (n = 19)	Control (n = 51)	*P* Value
A. Analysis of first CBC			
ATN (cells/mm^3)	2118 ± 1441*	3044 ± 2729	.20
ATI (cells/mm^3)	315 ± 330	251 ± 519	.72
I:T	0.25 ± .26	0.10 ± .22	.06
ATN			
Outside Mouzinho reference range	16†	20	.52
Outside Manroe reference range	58	51	.64
ATI outside reference range	5	8	.54
I:T outside reference range	58	14	.002
≥1 Abnormality			
Mouzinho reference range	58	27	.03
Manroe reference range	74	55	.16
B. Analysis of serial CBCs‡			
≥1 Abnormality			
Mouzinho reference range	94	41	<.001
Manroe reference range	100	90	.210

* Values are means ± SD.
† Values are percentages.
‡ GBS n = 16; control group n = 39.

Of the GBS-infected neonates, 58% had ≥1 abnormality in neutrophil values detected on the first CBC using the data of Mouzinho et al[32] vs 27% of controls ($P = .03$). Corresponding percentages using the reference ranges of Manroe et al[24] (74% vs 55%, respectively) are not significantly different. Thus specificity for the initial CBC using the reference ranges of Mouzinho et al[32] was significantly greater than that of Manroe et al,[24] 73% vs 45% ($P = .003$ by McNemar's test).

When we analyzed serial CBC data using the presence of ≥1 abnormality, there was no significant difference in sensitivity between the two reference ranges (100% for Manroe et al,[24] 94% for Mouzinho et al[32]; $P = 1.0$). However, as shown in Table 4, the specificity was significantly better using the data of Mouzinho et al[32] vs Manroe et al,[24] 59% (100% − 41%) vs 10% (100% − 90%) ($P < .0001$).

Considering the reference ranges of Mouzinho et al[32] and estimating prevalence of early-onset GBS at 1%, the PPV and NPV for the initial CBC were 2.1% and 99.4%, respectively. Corresponding values for the Manroe et al[24] reference ranges were 1.3% and 99.3%. When serial counts were used, the PPV and NPV for the Mouzinho et al[32] reference ranges were 1.5% and 99.7%, respectively; corresponding values for Manroe et al[24] were 1.1% and 100%, respectively.

DISCUSSION

Sepsis in the VLBW neonate can be a devastating problem, leading to considerable morbidity and mortality.[1,2] The inability to adequately exclude the diagnosis of neonatal sepsis, on the other hand, can result in unnecessary and prolonged exposure to antibiotics. Thus, laboratory tests that assist the clinician in the diagnosis of infection in VLBW neonates have

considerable relevance. In 1979, we[24] reported reference ranges for circulating neutrophils in neonates ≤30 days and demonstrated their utility in confirming the presence or absence of neonatal sepsis.[23,25,27] More recently, we and others[27–31] observed the frequent occurrence of neutropenia in otherwise healthy VLBW neonates when the 1979 reference ranges were used. Because of these findings, we examined the reference ranges for circulating neutrophils in VLBW neonates ≤30 days, a group of neonates whose survival had increased significantly over the ensuing 14 to 15 years.[32] In this study, we observed a broader distribution for ATN values in the first 72 hours after birth. Thus, although we revised these reference ranges, those for ATI and I:T values were unchanged. Because of the small number of infants with sepsis in that study,[32] no conclusions regarding the usefulness of the revised reference ranges in the diagnosis of infection could be made. Thus, we undertook the present study to assess whether there are differences in the usefulness of the previous[24] and revised[32] reference ranges reported by us in establishing or ruling out a diagnosis of sepsis in the VLBW neonate.

In the present report, the sensitivity for diagnosing neonatal sepsis is reduced using the more BW-specific ATN reference ranges of Mouzinho et al[32] in certain circumstances. This might have been anticipated since Mouzinho et al[32] observed a decrease in the lower limit of normal for ATN values throughout the first 60 hours after birth. Because GBS sepsis and pneumonia are most common during the first 72 hours after birth, one might expect that differences in the sensitivity of neutrophil values between the two reference ranges would be most apparent in these neonates in whom neutropenia has been shown to occur frequently.[24–26] However, in the presence of early-onset GBS infection, at least one neutrophil value was abnormal in 58% to 74% of initial CBCs and 88% to 94% of second CBCs, the latter demonstrating the value of serial CBC determinations, and there was no significant difference in sensitivity between the reference ranges of Mouzinho et al[32] and Manroe et al.[24]

The occurrence of neutropenia in association with early-onset sepsis has been reported previously by us[25] and others.[3,26] Christensen[35] has suggested that this is likely attributable to bone marrow depletion as well as a diminished capacity for accelerated neutrophil production by the preterm, VLBW neonate. This, however, appears to be limited to the first week after birth, because neutrophilia and elevated ATI and I:T values are commonly observed after that time.[32] The current study, in which most instances of neutropenia occurred in the first 72 hours after birth, supports our previous observations. Furthermore, it is notable that late-onset infection with coagulase-negative staphylococci is rarely associated with neutropenia, whereas 15% to 20% of episodes of late-onset Gram-negative infection presented with or developed neutropenia. The reason for this difference is not known, but it may be related to the virulence of the infecting organism.

The differences in sensitivity that were observed between the two reference ranges may have limited clinical importance. For example, when all organisms were considered during the 73 to 720-hour period, the difference in sensitivity was minimal, 82% (Manroe et al[24]) vs 77% (Mouzinho et al[32]). However, the greater specificity shown for the Mouzinho et al[32] reference ranges may be clinically relevant. We found that control neonates, matched with cases of early-onset GBS infection, were much less likely to have abnormal neutrophil values according to the Mouzinho et al[32] reference ranges than with those of Manroe et al[24] (first CBC, $P = .004$; serial CBCs, $P < .0001$). The enhanced specificity with the revised reference ranges shown for early-onset GBS sepsis, especially when considered in light of the very high sensitivity associated with serial neutrophil values, may be useful in determining the duration of treatment in neonates begun on antibiotics, but in whom cultures are either sterile or suggestive of contamination after 48 hours of treatment.

The results of this study provide support for the utility of assessing circulating neutrophil values in the diagnosis of sepsis in VLBW neonates, although not all investigators consider the laboratory differentiation of neutrophil values to be a reliable test in the neonate.[36] In a large group of VLBW neonates with proven sepsis during a 7-year period, there was only a 6% and 11% likelihood of all neutrophil values being normal, using the reference ranges of Manroe et al[24] and Mouzinho et al,[32] respectively. Estimations of PPV and NPV indicated that PPV is fairly low while NPV is very high (90%-100%). As noted above, these values are dependent on prevalence, and thus in institutions with a higher prevalence of early-onset GBS, values for PPV and NPV can be different; as prevalence increases, PPV also increases but NPV decreases (if sensitivity and specificity are constant).

Antibiotic therapy should not be withheld in neonates considered at risk for sepsis while awaiting the results of an initial CBC, because these neutrophil values may frequently be normal. Indeed, this appears to reflect current practice in most nurseries. It is reassuring that completely normal neutrophil values on serial CBCs were unlikely to occur in septic VLBW neonates. Conversely, normal values were observed in the majority of matched-controls. Based on these findings and our experience, discontinuation of antibiotics after 48 hours[37] seems appropriate in infants with normal serial neutrophil values (Mouzinho et al[32] reference ranges) and clinical information suggesting the absence of infection. We conclude that the revised reference ranges of Mouzinho et al[32] can be used to better assess circulating neutrophil values in the VLBW neonate with possible sepsis, generally by decreasing the number of false positives compared with the Manroe et al[24] reference ranges.

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