RESEARCH ARTICLE

Determining Normative Values for Cerebrospinal Fluid Profiles in Infants

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ABSTRACT

BACKGROUND: Previous studies of reference values for cerebrospinal fluid (CSF) profiles have been limited by small sample size and few exclusion criteria.

OBJECTIVE: To determine age-specific normative CSF white blood cell count (WBC), glucose, and protein values in infants ≤90 days old.

METHODS: Performed a retrospective cross-sectional study of infants ≤90 days old who had a diagnostic lumbar puncture between 2008 and 2016. Infants with bacterial meningitis, bacteremia, UTI, positive CSF herpes simplex virus polymerase chain reaction (PCR) result, traumatic lumbar puncture, ventriculoperitoneal shunt, prematurity, recent seizure, previous antibiotic use, and history of a complex chronic condition were excluded for calculations to determine normative values. Data on demographics and CSF values (WBC with differential, protein, glucose, enterovirus PCR) were collected. CSF values were compared by age and by enterovirus PCR results using Kruskal–Wallis and Wilcoxon rank tests.

RESULTS: A total of 1029 out of 2000 patients were included and divided into 3 age groups: 0 to 28 days, 29 to 60 days, 61 to 90 days. CSF WBC values were significantly greater for 0- to 28-day old infants (median: 3, 95th percentile: 14) than for 29- to 60-day and 61- to 90-day old infants (median: 2 and 2; 95th percentile: 7 and 11, respectively) ($P < .001$). With each month of life, the median CSF protein significantly decreased and glucose significantly increased. In the CSF WBC differential, monocytes were found to be prevalent.

CONCLUSION: We determined age-specific normative components for CSF profile values for infants 0 to 90 days.

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The incidence of serious bacterial infections in febrile infants ≤90 days old is estimated to be 7.0% to 13.5%.[1,2] Prompt identification and treatment is essential to avoid poor outcomes. In current clinical practice, a lumbar puncture (LP) is often executed as part of the workup for neonates (≤28 days) presenting with fever (≥38°C) without a source.[3] Although substantial variation in practice exists, LPs are also performed on infants >28 days on the basis of clinical suspicion for meningitis after reviewing history, physical examination, and laboratory results.[4] With each LP, cerebrospinal fluid (CSF) culture is sent along with the standard CSF profile composed of CSF white blood cell count (WBC), CSF red blood cell (RBC), CSF glucose, and CSF protein. Physicians use these results to refine the differential diagnosis and determine the need for empirical antibiotics and admission.

Researchers in several studies have investigated normative values for CSF profile components.[5–11] However, these studies are limited because of either their sample size or less stringent exclusion criteria. Only 1 has a sample size greater than 1000 infants, but it did not exclude patients who received antibiotics before culture collection or those with complex chronic conditions.[11] The objective with this study is to analyze the CSF profiles of infants from 0 to 90 days old to determine reference CSF values using more stringent exclusion criteria. Similar to previous studies, our population includes infants who clinically required a lumbar puncture for fever or other symptoms concerning for meningitis. It would be unethical to impose the risks of the procedure on infants without clinical indication.

METHODS

Study Design

This retrospective study was performed at a single academic health system composed of 3 freestanding children's hospitals, located in an urban area in the Southeast. Two of the hospitals are tertiary care centers, whereas the third is a community hospital. Typically, the emergency departments (EDs) at these sites see >200 000 patients yearly.

Permission to conduct this study was obtained from the institutional review board of this organization. Waiver of informed consent was granted.

Participants

Infants from 0 to 90 days old who presented to either the ED or inpatient setting between the years of 2008 to 2016 and had a diagnostic lumbar puncture for clinical suspicion of infection were eligible for this study. A total of 2000 charts were individually reviewed for the following exclusion criteria: positive (CSF, blood, or urine) culture treated with antibiotics, positive CSF herpes simplex virus polymerase chain reaction (PCR) result, traumatic tap (RBC >500),[12,13] history of prematurity (<37 weeks),[14–16] ventriculoperitoneal shunt, seizure 48 hours before LP,[14–16] and preprocedure antibiotic use (up to 24 hours beforehand).[17,18] In an effort to isolate CSF values truly reflective of the healthy infant, patients with a history of complex chronic condition, defined by Feudtner's classification system, were also excluded because little is known about CSF values in this population.[19] Finally, if CSF protein or glucose was reported as 0, these values were excluded because they were likely entered in error.

Deidentified data were entered into Research Electronic Data Capture,[20] a secure tool for data collection. Demographics, length of stay, presence of

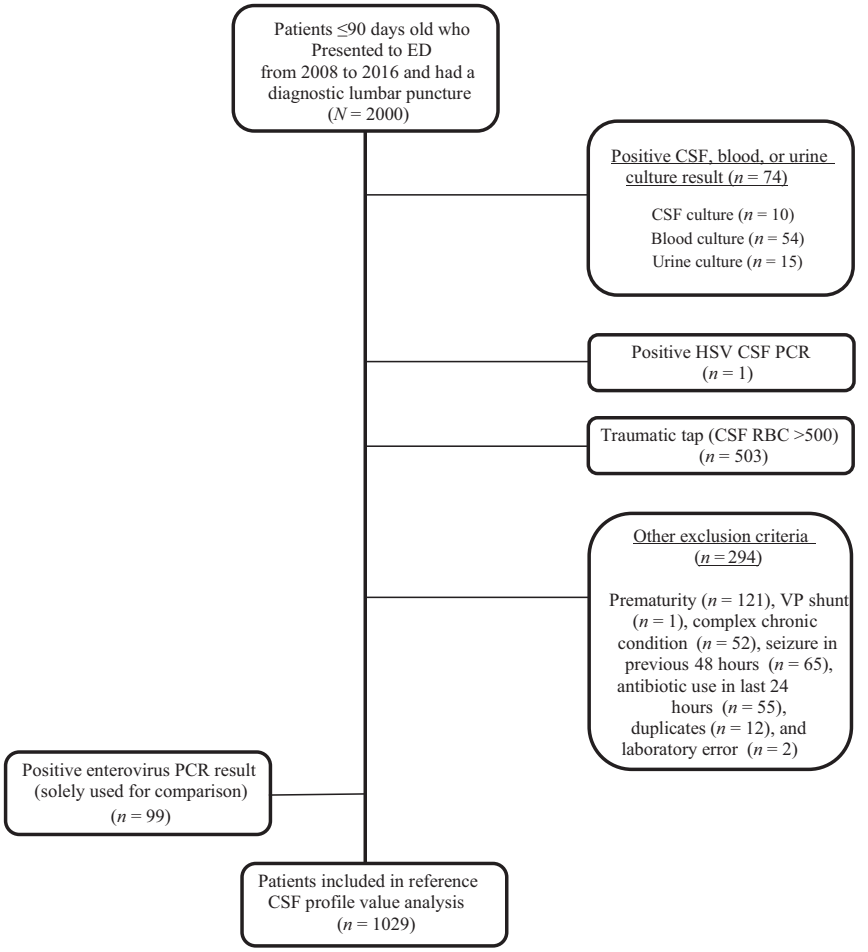

FIGURE 1 Study population: flowchart of patients excluded from our study. *Some patients included in the "other exclusion criteria" category met >1 exclusion criteria. VP, ventriculoperitoneal.

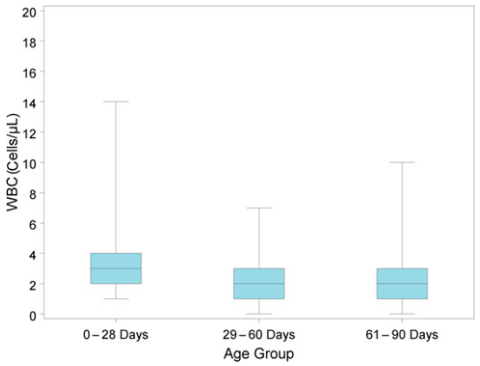

FIGURE 2 Box and whisker plot CSF WBC counts based on age groups. Demarcated by the whiskers, the minimum and maximum represent the 5th and 95th percentiles, respectively.

fever (temperature of ≥38°C either by report or documented in the ED), CSF profile (WBC with differential, RBC, glucose, and protein), and CSF enterovirus PCR results were collected. Patients with positive enterovirus PCR result were not used to determine normative values but were later used in a subset analysis to compare CSF values with those in the not-tested and negative groups. Because enterovirus PCR was not performed for every patient, this subset analysis was also performed to compare CSF values between the negative and not tested groups. It is plausible that some patients in the not tested groups may have had enterovirus meningitis. To use data from both the negative and not tested groups to define normative values, this analysis was completed to ensure there was no significant difference between the 2 groups.

Statistical Analysis

The data obtained were divided into 3 categories based on the age of the patient: 0 to 28 days, 29 to 60 days, and 61 to 90 days. These age cutoffs are commonly employed in risk assessment guidelines to determine management recommendations.[21] In addition, using these age groups allows for easy comparison with previously conducted studies.[7,9,11]

SPSS (IBM SPSS Statistics, IBM Corporation) was used for statistical analysis. Mean, median, interquartile range (IQR), and the 95th percentile of CSF WBC, CSF WBC differential, CSF protein, and CSF glucose were calculated for each age group. The 5th percentile was computed for CSF WBC and glucose. These values were chosen on the basis of clinical pertinence as well as for ease of comparison with previous studies in which researchers investigated normative CSF values. The Kruskal–Wallis test was used to compare CSF profile values among the 3 age groups. Wilcoxon rank test was used to compare CSF WBC values for those with a negative enterovirus PCR versus not tested, positive versus not tested, and positive versus negative.

RESULTS

Of the 2000 patients identified, 1029 (51%) remained after applying the aforementioned exclusion criteria and removing the patients with a positive CSF enterovirus PCR result for calculations to determine normative CSF profile values (Fig 1). There were slightly more boys (54.2%) than girls (45.8%). The majority of patients presented with either reported or documented fever (73.7%). The average length of stay in the hospital was 2.3 days.

CSF WBC Results

The median, IQR, and 5th and 95th percentiles of CSF WBC are graphically presented in Fig 2. The median CSF WBC for the 0- to 28-day age group (3 cells/μl) was statistically higher than the CSF WBC for the 29- to 60- and 61- to 90-day age groups (Table 1). For all age groups, patients who tested negative for CSF enterovirus PCR did not have significantly different CSF WBC compared with those who were not tested. However, both the enterovirus negative and not-tested group had a significantly lower CSF WBC

TABLE 1 Summary of CSF Values by Age Strata

CSF Value	0–28 d, *n* = 559	29–60 d, *n* = 390	61–90 d, *n* = 80	*P* value
WBC, cells/μL				
Median (IQR) [95th percentile]	3 (2–4) [14]	2 (1–3) [7]	2 (1–4) [11]	<.001
RBC, cells/μL				
Median (IQR) [95th persentile]	3 (1–38) [316]	1 (0–23) [244]	1 (0–12.5) [218]	.002
Protein, mg/dL				
Median (IQR) [95th percentile]	67 (53–83) [119]	50 (40–64) [88]	41.5 (33–49.5) [74.5]	<.001
Glucose, mg/dL				
Median (IQR) [95th percentile]	42 (38–47) [59]	45 (40–50) [61]	48.5 (43.5–54.5) [67.5]	<.001

Patients with positive herpes simplex virus PCR and CSF enterovirus PCR results were excluded.
Groups with the same letter are not statistically different from one another at the *P* value = .01 level.

than the enterovirus-positive group (Table 2).

CSF Protein and Glucose Results

CSF protein was highest for the 0- to 28-day age group. There was a significant decrease in CSF protein with each consecutive age group. The opposite trend was seen in CSF glucose. With each subsequent age group, there was a statistically significant increase in glucose (Table 1). The 5th percentile of CSF glucose for each age group was calculated as follows: 0 to 28 days, 34 mg/dL; 29 to 60 days, 35 mg/dL; and 61 to 90 days, 40 mg/dL.

CSF WBC Differential

In both the enterovirus negative and not tested group, the majority of cells were composed of monocytes across all age groups. Patients who tested positive for enterovirus had a lower percentage of lymphocytes and a significantly higher percentage of neutrophils compared with those who tested negative or were not tested for enterovirus (Supplemental Table 4).

DISCUSSION

In our study, we defined normative values for each component of the CSF profile, including WBC, WBC differential, glucose, and protein, for infants ≤90 days old. These findings contribute to the current available literature. We confirmed that CSF WBC and protein decrease with age whereas CSF glucose increases with age. We also demonstrated a CSF monocytic prevalence throughout all age groups. This study includes the largest cohort of infants after applying strict exclusion criteria using manual chart review. Overall, it is the second largest in which normative values for infants are investigated. On the basis of our literature review, it is also the second in which CSF WBC in the 61- to 90-day age group is studied.

To determine reference values for CSF WBC, several factors known or suspected to alter the CSF profile were excluded, including positive CSF enterovirus PCR result. Although researchers have found that 30% of infants may not develop CSF pleocytosis with an enterovirus central nervous system infection, the majority of infants still do.[22] All of our patients did not have a CSF enterovirus PCR performed (11% were tested and negative). We found no statistically significant difference between the CSF WBC in the enterovirus negative group versus the not tested group; however, the 95th percentile for the enterovirus negative group was higher than that of the not tested group. We speculated that physicians may have been prompted to test those with slightly higher CSF WBC counts. It is also possible that these infants had a viral infection other than enterovirus. Without a noteworthy difference between the negative and not tested groups, both subsets were used to calculate normative data. Because of similar findings, this approach was also employed in the largest multicenter study investigating CSF reference ranges by Thomson et al[11] (∼14% of the study population was tested and negative for enterovirus).

For our overall analysis, the median CSF WBC was highest in the 0- to 28-day-old group and significantly decreased with each subsequent age group ($P < .001$). Despite being statistically different, the actual change in medians and 95th percentiles were not clinically compelling. In addition, the values themselves support using lower thresholds to define pleocytosis in clinical practice.[9] Our findings are analogous to previously published studies, from which results are summarized in Table 3. Ahmed et al[10] was the first to incorporate viral PCR testing to exclude patients while investigating baseline CSF values in infants. However, they only focused on the 0- to 30-day age group and had a small sample size of 108. Following, Byington et al[7] was the first study to only include infants with negative CSF enterovirus PCR testing. Despite not having CSF enterovirus PCR testing for all of the infants in our study, the 95th

TABLE 2 Summary of CSF WBC by Enterovirus Negative versus Not Tested Versus Enterovirus Positive

Age Group	CSF WBC	Enterovirus Positive ($N = 99$)	Enterovirus Negative ($N = 226$)	Not Tested ($N = 804$)	P (Negative Versus Not Tested)	P (Positive Versus Negative)	P (Positive Versus Not Tested)
0–28 d ($n = 612$)	Median	64.5	3	3	.016	<.001	<.001
	IQR	3–541	2–6	2–4	—	—	—
	95th percentile	1925	24	12	—	—	—
29–60 d ($n = 432$)	Median	97	2	2	.885	<.001	<.001
	IQR	4, 345	1, 3	1, 3	—	—	—
	95th percentile	1208	14	7	—	—	—
61–90 d ($n = 85$)	Median	90	2	2	.368	.005	<.001
	IQR	72–116	1–6	1–3	—	—	—
	95th percentile	156	26	6	—	—	—
Overall	Median	85	3	2	.061	<.001	<.001
	IQR	4–415	1–4	1–4	—	—	—
	95th percentile	1706	21	9	—	—	—

Units for CSF WBC are cells/μL. Groups are considered statistically significant if $P < .01$. —, not applicable.

TABLE 3 Summary of Results from Previous Studies Investigating Reference Values for CSF WBCs in Infants

Author	Year	Age, d	Population Size	CSF WBC cells/μL Mean (Median)	CSF WBC Upper Limit Cells/μL
This Study	2021	0–28	559	5.7 (3)	95th percentile = 14
		29–60	390	3.0 (2)	95th percentile = 7
		61–90	80	7.6 (2)	95th percentile = 11
Thompson et al[11]	2018	0–28	3467	5.5 (4)	95th percentile = 16
		29–60	4029	3.6 (2)	95th percentile = 11
Byington el al[7]	2011	1–28	278	6.1 (5.0)	92nd percentile = 18
		29–60	318	3.1 (3)	92nd percentile = 8.5
		61–90	81	3.0 (3)	92nd percentile = 8.5
Kestenbaum et al[9]	2010	0–28	142	9.2 (3)	95th percentile = 19
		29–56	238	3.1 (2)	95th percentile = 9
Ahmed et al[10]	1996	0–7	17	15.3 (6)	90th percentile = 18
		8–14	33	5.4 (6)	90th percentile = 10
		15–21	25	7.7 (4)	90th percentile= 12.5
		22–30	33	4.8 (4)	90th percentile = 8
Bonadio et al[8]	1992	0–30	35	11.0 (8.5)	90th percentile = 22
		31 –60	40	7.1 (4.5)	90th percentile= 15

Byington et al calculated the upperbound as the third quartile + 1.5 IQR ~92nd percentile.

percentile for our CSF WBC in the 0- to 28-days cohort was less than their upper bound (~92nd percentile). Upper limits for the other age groups were comparable.

For CSF protein concentration, we uncovered values resembling the findings in Thomson et al[11] for the 0- to 28- and 29- to 60-day-old subsets. This occurred even though their study did not exclude patients pretreated with antibiotics, which has been shown to decrease protein and increase glucose concentrations.[18] Also seen in our study, Shah et al[23] demonstrated a decrease in CSF protein concentration based on age. Using 2-week age subsets, they found slightly lower concentrations when specifically looking at the 29- to 42- and 43- to 56-day age ranges (95th percentile: 89 mg/dL, 82 mg/dL respectively). Byington et al[7] inspected the CSF protein concentration in the 61- to 90-day age group in their study. Their upper bound (approx. 92nd percentile) was 71 mg/dL and similar to our results.

CSF glucose concentrations in our study population significantly increased with each age group ($P < .001$), which complements findings published by Thomson et al.[11]

As for the CSF WBC differential, researchers in previous studies did not study individual cell lines so direct comparison was difficult. We found that there was a monocytic predominance in those presumed to be healthy. The percentage of neutrophils was significantly lower in those with negative enterovirus PCR and not tested in comparison with those who were positive for enterovirus (P value $<.001$). A significant increase was not seen in the percentage of lymphocytes in those positive for enterovirus, showing that those with enterovirus meningitis may present with an increased neutrophil count.

This study has several limitations. First, this is a single-center study, so our results would not be as generalizable as a multicenter study. Second, since only a small sample of infants were tested for enterovirus (11%), it is possible that some patients had another unidentified viral cause of meningitis. This could lead to falsely elevated upper limits in our predicted CSF WBC range. However, our CSF WBC values were similar if not lower than those reported in previous studies. Also, the general trends seen in our study were similar to results in previous publications and confirm the findings reported by Thomson et al,[11] which had a larger patient population. We feel that the normative values found in our study contribute to current data used to interpret results of CSF profiles in infants presenting to the ED with concern for a central nervous system infection.

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