RESEARCH ARTICLE

Evaluation for Neonatal HSV in Infants Undergoing Workup for Serious Bacterial Infection: A 5-Year Retrospective Review

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ABSTRACT

OBJECTIVES: To describe the characteristics of infants evaluated for serious bacterial infection, focusing on empirical testing and treatment of herpes simplex virus (HSV) and describe the characteristics of HSV-positive patients.

METHODS: We included infants aged 0 to 60 days undergoing evaluation for serious bacterial infection in the emergency department. This descriptive study was conducted between July 2010 and June 2014 at a tertiary-care children’s hospital. Eligible patients were identified on the basis of age at presentation to the hospital and laboratory specimens. Infant characteristics, symptoms on presentation, and laboratory workup were compared between HSV-positive and HSV-negative patients by using the 2-sample t test or the Wilcoxon rank test.

RESULTS: A total of 1633 infants were eligible for inclusion, and 934 (57.2%) were 0 to 28 days of age. HSV was diagnosed in 19 infants, 11 of whom had disseminated disease. Compared with those without HSV, HSV-positive infants were younger, less likely to be febrile and to present with nonspecific symptoms, and more likely to have a mother with HSV symptoms (P < .05). Testing from all recommended locations was only performed in 22% of infants. Infants tested or empirically treated with acyclovir had a longer median length of stay compared with children who were not tested or treated (P < .01).

CONCLUSIONS: The absence of fever should not preclude a workup for HSV in neonates, and when a workup is initiated, emphasis should be placed on obtaining samples from serum, cerebrospinal fluid, and surface specimens. Physicians may benefit from a guideline for evaluation of HSV with specific guidance on high-risk features of presentation and recommended testing.

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Dr Brower conceptualized the study, directed the literature review process, drafted the initial manuscript, and reviewed and revised the manuscript; Drs Wilson and Schondelmeyer contributed to the study development, conducted the literature review process, drafted the initial manuscript, and reviewed and revised the manuscript; Drs Murtagh-Kurowski and Courter provided crucial clinical expertise during study development and critically reviewed and revised the manuscript; Dr Shah conceptualized and directed the study development, provided clinical expertise in the literature review process, and reviewed and revised the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Neonatal herpes simplex virus (HSV) infection carries a high risk of mortality and, among survivors, morbidity, including developmental delay and seizures. Accurate and timely diagnosis, which is necessary to reduce mortality, is complicated by the range of clinical presentations, which can include few or no signs, symptoms, or characteristic laboratory findings.

Although diagnosis in infants with skin-eye-mucosal (SEM) disease may be immediately apparent on the basis of the presence of vesicles, other manifestations of HSV may be more difficult to identify. Infants with disseminated disease, for example, can present with fulminant sepsis and disseminated intravascular coagulation or simply with hypothermia and lethargy. Similarly, infants with isolated central nervous system (CNS) HSV can present with few nonspecific clinical and laboratory findings, especially early in the disease.

Guidelines for the evaluation of serious bacterial infections (SBIs) in infants aged 0 to 60 days presenting with fever and signs of illness are well established, but a workup for HSV is at the discretion of the treating physician. The American Academy of Pediatrics provides recommendations for the diagnosis of HSV, but there is controversy concerning which infants merit evaluation, which leads to variation in testing and empirical treatment. The epidemiology of neonatal HSV may be changing because of increased risk of first-time genital HSV infections in younger women of childbearing age, which poses the highest risk of vertical transmission. The most recent estimates indicate that the incidence of disease is on the rise and currently thought to occur in 4.5 patients in 10,000 births. Thus, it is important to establish an understanding of how clinicians approach identifying infants at a high risk of infection while balancing the potential harms of overtreatment with the risk of delayed diagnosis. In view of the potential changing epidemiology of neonatal HSV infection, it is important to better understand the characteristics of infants being evaluated for HSV.

Our goals in this study were to (1) describe the characteristics of infants evaluated for SBI at our hospital over a 5 year period, focusing on empirical testing and treatment of HSV; (2) describe the characteristics of HSV-positive patients at our hospital over the same time period; and (3) compare HSV-negative infants who were empirically tested and treated for HSV with those who were not.

**METHODS**

**Study Design and Setting**

We conducted a descriptive study of young infants undergoing evaluation for SBI, with a focus on HSV evaluation, between July 2010 and June 2014 at a Midwest tertiary-care children’s hospital. This time period was chosen because it preceded implementation of a clinical pathway that standardized diagnostic evaluation of infants receiving lumbar puncture. The hospital has ~120,000 emergency department (ED) visits and 35,000 admissions annually. This study was approved by the institutional review board.

**Sample Description and Data Collection**

We included infants aged 0 to 60 days undergoing evaluation for SBI in the ED. The evaluation for SBI was defined on the basis of the local febrile neonate guideline and the intent of the medical team as the presence of blood, urine, and cerebrospinal fluid (CSF) cultures for infants 0 to 28 days of age and the presence of blood and urine cultures for infants 29 to 60 days of age. We identified eligible patients on the basis of age at presentation to the hospital and at collection of laboratory specimens. We performed an initial electronic medical record (EMR) review to determine if the infant had undergone evaluation for SBI. For infants with multiple visits during the study period, each visit was included independently for data extraction. Additionally, we reviewed all positive HSV polymerase chain reaction (PCR) test results during the study period to ensure that no patients with HSV were missed because of an incomplete initial diagnostic evaluation in the ED or because the initial workup was performed at an outside facility but HSV testing was performed after transfer.

We created a standardized data collection tool using a secure data entry platform. For infants meeting inclusion criteria, we completed detailed EMR data extraction to obtain data on HSV status, age and symptoms at presentation, sex, gestational age at delivery, HSV exposure history, presence of complex chronic conditions (CCCs), laboratory testing, acyclovir use, length of stay (LOS), and record of extravasation. For this study, we used a previously developed definition for CCCs that includes congenital anomalies in the following categories: neurologic, cardiac, pulmonary, gastrointestinal, gastrointestriary, endocrine or metabolic, and genetic. For the EMR abstraction, we classified symptoms at presentation as follows: respiratory symptoms included cough, congestion, cyanosis, difficulty breathing, and apnea; neurologic symptoms included seizure, altered mental status, an abnormal neurologic examination, excessive sleepiness or fussiness, and irritability; skin and soft tissue symptoms included vesicular or petechial rash, blanching rash, abscess, mastitis, cellulitis, and bruising; and head, neck, eyes, ears, nose, and throat symptoms included otitis and conjunctivitis. The symptoms on presentation were not mutually exclusive, and neonates could have presented with multiple symptoms in a single category or different categories. For patients with HSV, we collected additional data on age at symptom onset, hours to acyclovir initiation, ICU admission, need for intubation or inotropic support, and death.

**Variables and Statistical Analysis**

For our first objective, we grouped infants by HSV status and, for those without HSV by age (0-28 days and 29-60 days). We defined SEM disease as a PCR test from only the skin, eyes, or mouth positive for HSV. CNS disease was defined as a CSF PCR test (with or without skin findings) positive for HSV. Disseminated HSV was defined as the presence of a positive serum PCR test result in addition to hepatitis, pneumonitis, disseminated intravascular coagulation, or other end-organ damage other than isolated
CNS manifestations (with or without skin findings). We classified infants as HSV-positive if they had any positive HSV PCR testing results or if they received a 21-day treatment course of acyclovir for presumed HSV. The remainder of the infants were classified as HSV-negative. Because HSV is a progressive illness if left untreated, we classified infants with no testing for HSV as HSV-negative at time of visit. HSV-positive and HSV-negative infants were compared on the basis of age at presentation, sex, gestational age at delivery, HSV exposure (maternal HSV symptoms or household contact with HSV), and symptoms or physical examination findings at presentation. Descriptive statistics were used to summarize types of testing performed and acyclovir use. We defined complete testing evaluation on the basis of published recommendations as HSV PCR testing from the CSF, serum, surface (conjunctiva, nasopharynx, or rectum), and vesicle or lesion (if present on examination) as well as hepatic transaminase testing.

For our second objective, we used descriptive statistics to summarize characteristics of infants diagnosed with HSV, including testing results and outcomes. For our third objective, we grouped HSV-negative infants on the basis of whether they underwent empirical testing or treatment of HSV, defined as undergoing any HSV PCR test or receiving ≥1 dose of acyclovir. We compared the following dependent variables between these 2 groups: (1) median LOS, (2) presence of intravenous extravasation, (3) development of acute kidney injury (AKI). AKI was defined as an absolute serum creatinine level ≥1.5 mg/dL or a rise in serum creatinine levels of 1.5 times the initial value.

Data were analyzed by using Stata 14.0 (Stata Corp, College Station, TX). Categorical variables were described by using percentages and compared by using Fisher’s exact test. Continuous variables were described by using means and SDs for normally distributed data and medians and interquartile ranges (IQRs) for skewed data. Analyses were completed by using the 2-sample t test or the Wilcoxon rank test.

### RESULTS

A total of 1633 infants were eligible for inclusion, and 934 (57.2%) were 0 to 28 days of age. Twenty-nine patients had multiple evaluations for SBI over the study period; 1 infant had 3 visits, and the remainder had 2 visits. Four of the 29 revisits were for infants with HSV who presented after completion of hospitalization and treatment of their initial HSV disease for a second evaluation of fever. Eleven of the 29 infants with revisits underwent evaluation for SBI because of a history of a previous positive blood culture result. Table 1 reveals the demographic variables and characteristics of infants evaluated for SBI. No infants with HSV had a previously diagnosed CCC. Of the HSV-negative infants, 16 had gastrointestinal conditions, 14 had cardiac conditions, 11 had genetic conditions, 10 had neurologic conditions, 6 had gastrointestinal conditions, and single infants had pulmonary, endocrine, or metabolic conditions.

HSV was diagnosed in 19 infants. Compared with those without HSV, HSV-positive infants were younger (mean: 11.3 vs 26.4 days;
<table>
<thead>
<tr>
<th>Case No.</th>
<th>SEM Disseminated</th>
<th>CNS Unknown(^a)</th>
<th>CNS Negative</th>
<th>CNS-Positive</th>
<th>CNS</th>
<th>Potential HSV(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td></td>
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<tr>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>13(^c)</td>
<td>14</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Age, d</td>
<td>At symptom onset</td>
<td>21</td>
<td>6</td>
<td>5</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>At presentation</td>
<td>28</td>
<td>8</td>
<td>5</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Symptoms or PE</td>
<td>HSV high risk</td>
<td>Rash, rash, scalp site</td>
<td>&lt;30°C, il, AMI, extensive bleeding</td>
<td>&lt;30°C, il, AMI, abnormal neurologic examination, excessive bleeding</td>
<td>AMI, il, likely maternal primary genital HSV</td>
<td>AMI, il, likely maternal primary genital HSV</td>
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<tr>
<td></td>
<td>Other</td>
<td>None</td>
<td>None</td>
<td>Difficulty breathing, poor feeding</td>
<td>Poor feeding, vomiting</td>
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<tr>
<td></td>
<td>Testing</td>
<td>CSF WBCs per mL</td>
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<td>4</td>
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<td>—</td>
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<td></td>
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<td>RBCs per mL</td>
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<td>7</td>
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<tr>
<td></td>
<td></td>
<td>Glucose, mg/dL</td>
<td>48</td>
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<td>Negative</td>
<td>—</td>
<td>—</td>
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<td>Lesion</td>
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<tr>
<td></td>
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<td>Other</td>
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<td>+ tracheal</td>
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<td></td>
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<td>Other, U/L</td>
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<tr>
<td></td>
<td>Hours to acyclovir</td>
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<td>4</td>
<td>17</td>
<td>7</td>
<td>14</td>
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<tr>
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<td></td>
<td></td>
<td>LOS, d</td>
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<td>15.8</td>
<td>16(^d)</td>
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<td>Intubation</td>
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<td>No</td>
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<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

\(^a\) Potential patients with HSV are those without positive HSV testing results but who received a full 21-d course of acyclovir for possible HSV.

\(^b\) All of the patients with disseminated HSV and CNS unknown status were too ill at presentation for lumbar punctures.

\(^c\) No CSF cell counts were available because of low CSF volume and traumatic lumbar puncture.

\(^d\) HSV high-risk symptoms and PE findings are based on common HSV symptoms in published case series of HSV-positive infants.

\(^e\) Probable maternal HSV based on signs and symptoms, but no access to maternal medical record to verify.

\(^f\) For each patient who died, the LOS is days in the hospital before death.

\(^g\) Neonatal AKI definitions were adapted from Selewski DT, Charlton JR, Jetton JG, et al. Neonatal acute kidney injury. *Pediatrics*. 2015;136(2). Available at: www.pediatrics.org/cgi/content/full/136/2/e463.

ALT, alanine aminotransferase; AMI, altered mental status; AST, aspartate aminotransferase; BAL, bronchoalveolar lavage; NR, not reported; PE, physical examination; RBC, red blood cell; seg, segmented neutrophil; WBC, white blood cell; —, not applicable.
P < .05) and more likely to have a mother with HSV symptoms (32% vs 0.8%, P < .05). On presentation to the ED, HSV-positive neonates were less likely to be febrile but more likely to present with nonspecific findings, including ill appearance, hypothermia, difficulty breathing, and poor feeding.

Characteristics of the specific HSV cases are presented in Table 2. All but one patient with SEM disease presented at ≤21 days of age. Although the overall numbers are small, patients with disseminated disease presented at a younger age than those with SEM or CNS disease. The average patient age in days for each disease type at presentation was 9.7 days for disseminated disease, 14.5 days for CNS disease, and 18 days for SEM disease. Of the 19 cases of neonatal HSV, all but 2 cases had positive PCR testing results. One of the infants with negative PCR testing results had a complete negative PCR evaluation but a rash that was concerning for cutaneous HSV, a mother with active oral HSV, and no CSF cell counts because of inadequate CSF volume. The second infant with negative PCR testing results had fever and poor feeding, with mononuclear CSF pleocytosis, but an inadequate CSF volume to obtain CSF PCR testing. Both infants were presumed positive for HSV and treated with 21 days of intravenous acyclovir. More than half of the HSV-positive infants had disseminated disease (n = 11); 5 were CNS-positive, and 3 had an unknown CNS status because of the lack of an adequate CSF volume (secondary to severe illness).

For infants without HSV, PCR testing was more prevalent in younger infants (0–28 days) compared with older infants (29–60 days) (Table 3). The CSF HSV PCR was the most common test obtained in all groups (31.7% of entire cohort). No HSV-negative older infants underwent complete testing for HSV per American Academy of Pediatrics recommendations. All infants with HSV received acyclovir; 39% of HSV-negative young infants and 11% of HSV-negative older infants were empirically started on acyclovir. The median number of acyclovir doses received was 44 (IQR: 11–64) for the HSV-positive infants. The young HSV-negative infants received a median of 2 doses (IQR: 2–3), whereas the older HSV-negative infants received a median of 3 doses (IQR: 2–4).

Acyclovir was initiated within 24 hours of presentation in 84% of infants with HSV. All 5 (26%) deaths occurred in infants with disseminated disease. Acyclovir was initiated >24 hours after presentation for 3 infants with nonspecific symptoms at presentation, including altered mental status and ill appearance. Four infants developed AKI, all with disseminated disease. Eight infants (42%) required endotracheal intubation, and 5 required inotropic support (26%) (Table 2). For HSV-negative infants, 33% were tested for HSV or empirically treated with at least 1 dose of intravenous acyclovir. When compared with infants not evaluated for HSV, infants who were tested or empirically treated had a longer median LOS (48 [IQR: 41.2–71.3] vs 42.6 [IQR: 39.3–50.3] hours; P < .01). There were no significant differences between the groups in terms of cases of extravasation or AKI.

**DISCUSSION**

In this 5-year retrospective chart review, we described the characteristics of infants evaluated for SBI and highlighted presenting historical and physical examination findings that should raise suspicion for HSV infection. We found that HSV-positive infants commonly presented without fever and with nonspecific symptoms, such as poor feeding and difficulty breathing, that might not necessarily prompt specific concern for HSV infection. For the 19 infants diagnosed with HSV at our institution over the study period, we found that a complete evaluation was not consistently performed, suggesting that physicians may benefit from a guideline for evaluation of HSV with specific guidance on high-risk features of presentation and recommended testing. Finally, we showed that HSV-negative infants who received empirical testing and treatment of HSV have a longer LOS than those not tested or treated. These findings contribute to a growing understanding of the epidemiology and management of this rare entity.

Features of HSV-positive infants identified in our study were consistent with the existing literature. Specifically, we found a similar patient age at presentation for each subtype of HSV in our cohort,8–35 similar historical factors, such as exposure to HSV,1 or a poorly healing scalp electrode site,6,9,27,36 similar physical characteristics,6,13 compared with other published reports. These findings support consideration of these characteristics as ways to risk-stratify infants for a more targeted approach to evaluation for HSV.36 In our study, we also highlight the multitude of reasons, other than fever, that prompt physicians to evaluate for SBI in infants.5,5 Of the 19 cases of neonatal HSV, only 7 infants (37%) presented to the ED with fever. Temperature instability in the form of hypothermia has been associated with disseminated disease, which was noted in 22% of the neonates with HSV in our cohort;
therefore, it is important that clinicians be aware of the potential for HSV to present in an afebrile child with nonspecific symptoms.\textsuperscript{37} Historical features, such as poor feeding\textsuperscript{6,8,39,34} or respiratory distress,\textsuperscript{6,13,38,40} have also been identified in other case series and should alert the physician to potential HSV disease in the appropriate context.

We found that only 22% of neonates with HSV had the appropriate complete evaluation for HSV performed, including transaminases and PCRs from all body fluids, which is similar to previous literature.\textsuperscript{21,38} No single test has the perfect sensitivity to identify all the variable presentations of HSV\textsuperscript{11–45}; therefore, it is imperative to attempt to obtain mucosal, serum, and CSF PCRs and transaminases to help identify early cases of HSV before infants become critically ill. In our case series, 5 HSV-positive infants died, 3 of whom had no CSF PCR testing performed. This highlights the difficulty in identifying neonates with potential HSV infection and the need for standardized high-risk criteria to help alert clinicians sooner to this disease process.

A substantial number of infants in our cohort >28 days of age underwent testing for HSV. Perinatally acquired HSV rarely presents after 28 days of age\textsuperscript{36,38}, nurse and physician education regarding the common age ranges of neonatal HSV disease may be an important part of any improvement effort to decrease unnecessary invasive tests and their associated financial costs and physical risks.\textsuperscript{37,41} We also noted that 11% of infants in this older, lower-risk age group received acyclovir, which increased their LOS and exposed those neonates to the potential harms associated with acyclovir.\textsuperscript{5,47,48} Although early recognition and treatment of HSV infection is paramount, clinicians should also be aware of the potential negative consequences of overtreatment and overtreatment.

Our study has several limitations. This was a retrospective study with data limited to those that were recorded in the EMR. We used a standardized chart abstraction tool to limit the subjectivity in data collection. Although our findings are from a single-center cohort, our hospital has a wide referral base and is the only children’s hospital within a 52-mile radius; therefore, in addition to patients from the community, we anticipate that the cohort may have included a larger proportion of patients with acute illness referred for a higher level of care. Because of the low incidence of disease, this single-center case series of neonates with HSV does not fully present the variability in presentation that has been reported in previous case series of HSV.

CONCLUSIONS

In this 5-year retrospective review of neonates undergoing evaluation for SBI, we identified typical and nonspecific clinical features that should alert physicians to evaluate and treat for neonatal HSV. Clinicians should remain wary of using fever as a primary criteria by which to identify infants at risk for HSV infection and should recognize the full breadth of testing required to evaluate for the 3 presentations of disease.

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