Predictors of Invasive Herpes Simplex Virus Infection in Young Infants

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abstract

OBJECTIVES: To identify independent predictors of and derive a risk score for invasive herpes simplex virus (HSV) infection.

METHODS: In this 23-center nested case-control study, we matched 149 infants with HSV to 1340 controls; all were ≤60 days old and had cerebrospinal fluid obtained within 24 hours of presentation or had HSV detected. The primary and secondary outcomes were invasive (disseminated or central nervous system) or any HSV infection, respectively.

RESULTS: Of all infants included , 90 (60.4%) had invasive and 59 (39.6%) had skin, eyes, and mouth disease. Predictors independently associated with invasive HSV included younger age (adjusted odds ratio [aOR]: 9.1 [95% confidence interval (CI): 3.4–24.5] <14 and 6.4 [95% CI: 2.3 to 17.8] 14–28 days, respectively, compared with >28 days), prematurity (aOR: 2.3, 95% CI: 1.1 to 5.1), seizure at home (aOR: 6.1, 95% CI: 2.3 to 16.4), ill appearance (aOR: 4.2, 95% CI: 2.0 to 8.4), abnormal triage temperature (aOR: 2.9, 95% CI: 1.6 to 5.3), vesicular rash (aOR: 54.8, (95% CI: 16.6 to 180.9), thrombocytopenia (aOR: 4.4, 95% CI: 1.6 to 12.4), and cerebrospinal fluid pleocytosis (aOR: 3.5, 95% CI: 1.2 to 10.0). These variables were transformed to derive the HSV risk score (point range 0–17). Infants with invasive HSV had a higher median score (6, interquartile range: 4–8) than those without invasive HSV (3, interquartile range: 1.5–4), with an area under the curve for invasive HSV disease of 0.85 (95% CI: 0.80–0.91). When using a cut-point of ≥3, the HSV risk score had a sensitivity of 95.6% (95% CI: 84.9% to 99.5%), specificity of 40.1% (95% CI: 36.8% to 43.6%), and positive likelihood ratio 1.60 (95% CI: 1.5 to 1.7) and negative likelihood ratio 0.11 (95% CI: 0.03 to 0.43).

CONCLUSIONS: A novel HSV risk score identified infants at extremely low risk for invasive HSV who may not require routine testing or empirical treatment.

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WHAT'S KNOWN ON THIS SUBJECT: Identification of young infants with invasive (central nervous system or disseminated) HSV infection is challenging, leading many well-appearing infants to be empirically tested and treated to prevent low-frequency, high-risk outcomes.

WHAT THIS STUDY ADDS: We derived a novel risk score for invasive HSV infection with 8 predictors (age, prematurity, seizure, appearance, abnormal temperature, vesicular rash, thrombocytopenia, and cerebrospinal fluid pleocytosis), which accurately identified infants at low risk of invasive HSV (negative likelihood ratio 0.11; 95% confidence interval: 0.03–0.43).

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Neonatal herpes simplex virus (HSV) infections, although infrequent,[1] often are serious and invasive, requiring prompt diagnosis and therapy to optimize outcomes.[2] However, infants with noncutaneous HSV infections often present with nonspecific symptoms (eg, irritability, poor feeding).[3] Identified risk factors for neonatal HSV infection include vaginal delivery, primary maternal HSV infection, and prolonged rupture of membranes.[4] However, previous studies are limited by small sample sizes and lack of comparison with noninfected infants and have not combined risk factors to develop risk stratification models.

Although >99% of infants evaluated for potential central nervous system (CNS) infection are not infected with HSV,[5] some guidelines recommend empirical HSV testing and treatment of infants in the first month of life undergoing cerebrospinal fluid (CSF) evaluation for potential infection.[6] Although even less common in the second month of life,[1] HSV infection remains a treatment consideration because of our inability to readily identify HSV-infected infants and the potential consequences of treatment delays.[2] However, a "test and treat-all" approach may introduce more harm than benefit; HSV testing is associated with prolonged hospitalization, increased acyclovir use, and higher costs.[7,8] Despite parenteral acyclovir being generally well tolerated, local extravasation can cause severe inflammation, phlebitis, and cutaneous necrosis.[9] Rapid infusion and administration to children with impaired renal function or those receiving other nephrotoxic medications can result in acute kidney injury.[9] Thus, the ability to risk stratify infants has the potential to optimize testing and treatment decisions. We conducted a multicenter nested case-control study to derive a clinical score using readily available clinical and laboratory parameters to stratify the risk of invasive HSV disease in infants ≤60 days of age undergoing evaluation for CNS infection.

METHODS

Study Design

We conducted a case-control study nested within a 23-center retrospective cohort study of infants presenting for emergency department (ED) care between January 1, 2005, and December 31, 2013 (Supplemental Table 5), as a planned substudy of the Pediatric Emergency Medicine Collaborative Research Committee (PEM CRC) of the American Academy of Pediatrics HSV study.[5] Participating sites spanned 16 US states and 2 Canadian provinces. Institutional review board approval was obtained from all participating sites with permission for data sharing.

Selection of Cases and Controls

The parent cohort included infants ≤60 days of age who had a CSF bacterial culture obtained within 24 hours after ED arrival to indicate a clinical concern for CNS infection or a positive HSV specimen collected from any source, including non-CSF locations. Infants with HSV infection without CSF collected within 24 hours were included to capture infants deemed too unwell to have a lumbar puncture performed within 24 hours of arrival and those initially thought to not require a lumbar puncture. Eligible encounters were identified by screening hospital electronic health records and microbiology laboratory databases. For both cases and controls, we only included the first eligible ED encounter.

Infants with HSV detected by polymerase chain reaction (PCR) or culture from any source were classified as cases. HSV serology was not used to define cases, because positive serology could reflect maternal exposure rather than neonatal infection.[10] All HSV cases were classified independently by 3 investigators (A.T.C., L.E.N., S.B.F.) into commonly employed HSV disease categories: skin, eyes, and mouth (SEM); CNS; or disseminated.[10] Disagreements were resolved by discussion and external consultation as required.

To identify risk factors, each HSV case was matched with 9 controls from the same study site in 3 control groups. The first control group (seasonal controls) consisted of the infants presenting to the ED closest in time (either before or after) to the case. The second control group (age controls) was selected from infants closest in chronological age to the case. Infants in the third control group (random controls) were randomly selected from the remaining eligible infants. We used multiple control groups to minimize the potential for bias and to permit evaluation of the consistency of our findings, thereby allowing us to assess the validity of our observed results.

Although eligible control infants did not have HSV infection, they may have had bacterial or other viral infections. Control infants who were untested or incompletely tested for HSV were assumed to be uninfected if HSV infection was not diagnosed during the hospital stay or through a repeat ED encounter within 7 days, on the basis of the short viral incubation period with expected progression to clinically evident disease in HSV-infected infants in the absence of treatment.

Data Collection

All study personnel reviewed the manual of operations and received standardized training in chart abstraction.[5,11,12] Structured chart reviews were conducted by site

investigator physicians or trained delegates. The primary data source was the ED medical record. Historical and physical examination findings were abstracted as documented by treating clinicians. If discrepancies were noted in the medical record, a hierarchy was used for abstraction, deferring to the note of the most senior practitioner. For the following factors, lack of documentation was interpreted as being absent: rash, seizure at or before presentation, intubation, ICU admission, or death. If not included in the ED note, historical data concerning maternal HSV infection or maternal intrapartum fever were abstracted from any section of the medical record. We defined CSF pleocytosis as a white blood cell (WBC) count >15 cells per mm^3 for infants ≤28 days and ≥10 cells per mm^3 for infants >28 days.[13]

Outcome Measures

Our primary outcome was invasive HSV disease, defined by the presence of CNS or disseminated disease. Although HSV disease is typically classified as SEM, CNS, or disseminated,[10] we combined CNS and disseminated disease into a single category referred to as "invasive" to align with front-line clinician decision-making. CNS disease was defined by the presence of a positive CSF HSV test result; disseminated disease by the presence of other end-organ damage including hepatitis (alanine aminotransferase [ALT] >1.5 times the upper limit of normal and/or evidence of disseminated intravascular coagulation)[14] or pneumonitis.15 Our secondary outcome was any HSV disease (SEM, CNS, or disseminated). SEM disease was defined by the detection of HSV from surface sources or blood, including isolated viremia, without visceral or CNS involvement.[10]

Statistical Analysis

Patient characteristics and outcomes were summarized by using medians and interquartile ranges (IQRs) for continuous variables and frequencies with percentages and 95% confidence intervals (CIs) for categorical variables. Differences were tested with the Wilcoxon and χ^2 tests, respectively. For the primary outcome of invasive HSV infection, we first fitted a backward stepwise ($P \leq .05$ removed) conditional logistic regression model including all case and control participants. Nineteen potential covariates were identified for assessment on the basis of previously demonstrated associations in the literature (Supplemental Table 6).[3,4,10,14–17] Next, we repeated the same regression analysis with each of our 3 control groups individually and with the secondary outcome of any HSV infection. Variables in the regression models were assessed for multicollinearity by using a variance inflation factor. To evaluate if the inclusion of infants with invasive bacterial infection (IBI; defined as bacteremia or bacterial meningitis) or infants tested for HSV would bias the association between the covariates and the outcomes of interest, we performed a sensitivity analysis excluding infants with IBI and including only those infants in whom HSV testing was performed, respectively.

To account for missing data in the covariates of interest and obtain CIs for our estimates, we combined multiple imputation approaches and assumed the data were missing at random. First, multiple imputation by fully conditional specification was performed by using SPSS Version 25.0 (IBM SPSS Statistics, IBM Corporation). One hundred data sets were imputed to make the minimum degrees of freedom of the parameter estimates >100.[18,19] The imputation model included the same covariates as the analytic model along with study site-level means of the following variables: triage temperature, hepatic transaminases, absolute neutrophil and platelet counts, serum glucose, and CSF cell counts and glucose. We specified logistic regression and predictive mean matching for imputation of categorical and continuous variables, respectively. Second, we used the mi estimate program in Stata software (Stata Corp, College Station, TX) on the multiple imputed data, and adjusted coefficients and standard errors for the variability between imputations according to the Rubin combination rules[20] and obtained the combined estimates along with 95% CIs.

We conducted penalized logistic regression to obtain adjusted odds ratios (aORs) for covariate of vesicular rash with sparse data. Identical covariates used for the conditional logistic regression model were included, along with a conservative 95% odds ratio interval limit for vesicular rash, on the basis of background information.

We derived an invasive HSV risk score by assigning points to each independent predictor variable on the basis of the estimated effect size from the regression model. We calculated the score's sensitivity, specificity, positive and negative likelihood ratios, and the area under the curve (AUC). The AUC of the risk score for predicting invasive HSV outcome was validated internally by using bootstrap resampling.[21] We also calculated score performance in infants with invasive HSV who lacked a rash at presentation. Only participants who had data available for the invasive HSV risk score calculation were included in this analysis. Analyses were conducted by using SPSS 25.0 and Stata 15.0.

RESULTS

Participants

We identified 149 cases of HSV disease, 112 identified from the parent cohort study and 37 through microbiology log reviews (Fig 1). Of the cases, 90 (60.4%) had invasive (46 disseminated, 44 CNS) disease and 59 (39.6%) had SEM (52 had mucocutaneous findings and 7 had isolated viremia). Twenty-seven infants (18%) were >28 days old. Invasive disease was seen predominantly in infants ≤28 days of age (80 of 90; 88.9%). The final diagnoses in the 81 (54.4%) infants who initially presented without any cutaneous findings included disseminated (n = 35, 43.2%), CNS (n = 27, 33.3%), and SEM (n = 19, 23.5%).

Of the 26 421 eligible infants in the parent cohort, we selected 1340 unique controls: 447 random, 446 age-matched (1 age-matched control was later deemed ineligible), and 447 seasonal. Eighteen (4.0%), 12 (2.7%), and 17 (3.8%) infants had an IBI in each control group, respectively. Among control participants, 39.1% (524 of 1340) and 24.2% (324 of 1340) were tested for HSV and received empirical acyclovir, respectively.

Invasive HSV Infection

Covariates independently associated with invasive HSV in multivariable regression analyses included age, prematurity, seizure before arrival, ill appearance, abnormal triage temperature, vesicular rash, thrombocytopenia, and CSF pleocytosis (Table 1). These variables all retained statistical significance in sensitivity analyses excluding controls with IBI (Supplemental Table 7) and in subgroup analyses of seasonal and random controls, although prematurity and thrombocytopenia were no longer associated with invasive HSV in the latter analysis, whereas neutropenia was found associated with invasive HSV in the analysis with seasonal controls alone. When controlling for age, the following were associated with invasive HSV infection: ill appearance, pyrexia or hypothermia at triage, presence of a vesicular rash, thrombocytopenia, and CSF pleocytosis. Sensitivity analyses including only infants in the control group in whom HSV testing was performed are displayed in Supplemental Table 8. Although missing data on transaminases precluded evaluation of the utility of aspartate aminotransferase (AST) or ALT for identifying disseminated disease, these parameters were obtained within 24 hours of admission in 35 of 46 (76.1%) of infants with disseminated HSV and were abnormal in 29 of 35 (82.9%).

Any HSV Infection

Variables independently associated with any HSV infection in multivariate analyses included age, illness duration >1 day, seizure before arrival, ill appearance, vesicular and nonvesicular rashes, CSF pleocytosis, and thrombocytopenia (Table 2).

Invasive HSV Risk Score

On the basis of the complete model for invasive HSV disease (Table 1), for which data were available for 867 (58.2%) infants, including 83 of 149 (55.7%) with HSV infection, 8 variables with individual point values of 0 to 4 were included in our HSV score (overall range of 0–17); Table 3. Infants with invasive HSV disease had a higher median risk score (6, IQR: 4–8) than those without invasive HSV infection (3, IQR: 2–4). The risk score's AUC for invasive HSV disease is 0.85 (95% CI: 0.80 to 0.91; Fig 2), and a cut-point of ≥3 has a sensitivity of 95.6% (95% CI: 84.9% to 99.5%), specificity of 40.1% (95% CI: 36.8% to 43.6%), positive likelihood ratio 1.60 (95% CI 1.47 to 1.70), and negative likelihood ratio 0.11 (95% CI: 0.028 to 0.43). Bootstrapping analysis (resampling 1000 times) on AUC of the score for predicting the invasive HSV outcome revealed a mean difference of 0.00022 (95% CI: −0.0015 to 0.0020) from the observed AUC. When using a cut-point of ≥3, 58% (401 of 689) infants without invasive HSV infection were misclassified as high risk, and 2 infants with invasive HSV infection (2 of 41, 4.9%) were

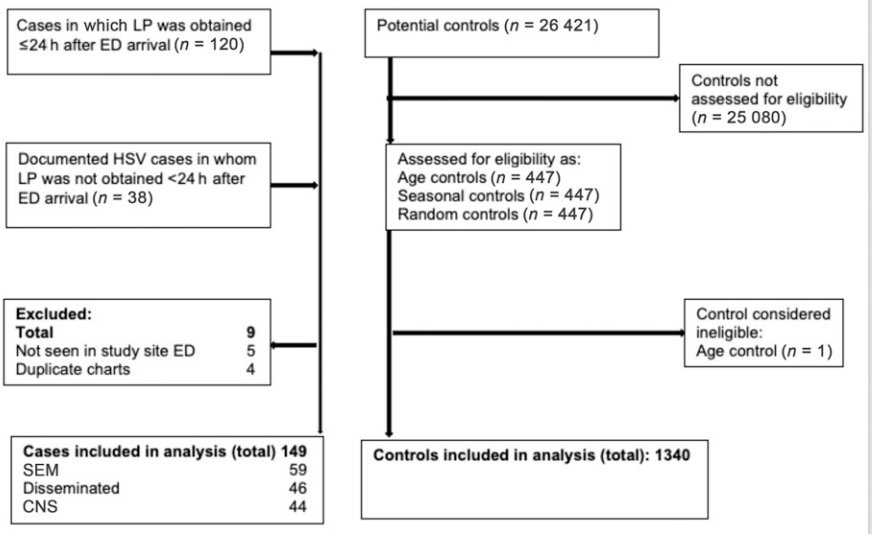

FIGURE 1
Subject identification LP, lumbar puncture.

TABLE 1 Multivariable Analyses with the Dependent Variable Being the Presence of Invasive HSV Infection

Candidate Predictor	All Controls, aOR (CI)	Seasonal Controls, aOR (CI)	Random Controls, aOR (CI)	Age-Matched Controls, aOR (CI)
Age				
<14 d	9.1 (3.4 to 24.5)	27.5 (7.1 to 106.0)	14.4 (4.8 to 43.4)	—
14–28 d	6.4 (2.3 to 17.8)	7.9 (2.3 to 27.6)	9.7 (3.0 to 31.7)	—
>28 d	Ref	Ref	Ref	—
Prematurity	2.3 (1.1 to 5.1)	—	—	—
Had seizure at home	6.1 (2.3 to 16.4)	17.6 (3.2 to 96.6)	5.8 (1.6 to 20.8)	3.4 (1.2 to 10.1)
Ill appearance	4.2 (2.0 to 8.4)	3.6 (1.5 to 8.6)	5.1 (1.8 to 14.4)	4.4 (1.8 to 10.8)
Abnormal triage temperature[a]	2.9 (1.6 to 5.3)	2.9 (1.2 to 7.0)	2.6 (1.2 to 5.9)	2.5 (1.2 to 5.0)
Vesicular rash[b]	54.8 (16.6 to 180.9)	113.5 (12.2 to 1059.2)	115.9 (8.8 to 1533.4)	29.4 (7.0 to 123.3)
Thrombocytopenia[c]	4.4 (1.6 to 12.4)	—	—	3.6 (1.1 to 11.2)
Neutropenia[d]	—	5.5 (1.5 to 20.8)	—	—
CSF pleocytosis[e]	3.5 (1.2 to 10.0)	4.7 (1.6 to 14.0)	4.3 (1.3 to 14.2)	2.9 (1.1 to 7.6)

Ref, reference group; —, the covariate was not statistically significant in the regression model.

[a] Includes triage temperature ≥38.0°C (100.4°F) or <36.4°C (97.5°F).

[b] The ORs and 95% CIs obtained from the penalized logistic regression with 95% previous limits on the OR scale (0.5 to 16) were 9.6 (95% CI: 4.4 to 21.1), 4.6 (95% CI: 2.0 to 10.7), 5.5 (95% CI: 2.5 to 12.2), and 3.8 (95% CI: 1.6 to 8.9) for the regression models including all controls, time controls only, age controls only, and random controls only, respectively.

[c] Platelet count <150 000 per mm^3.

[d] ANC <1000 cells per mm^3.

[e] CSF WBC >15 per mm^3 (≤28 d) or ≥10 per mm^3 (>28 d).

misclassified as low-risk by the HSV risk score (Table 4). Both were well appearing and normothermic; neither had seizures, neutropenia, or thrombocytopenia. In addition, 93% (42 of 45) of the infants with invasive HSV infection with missing data for ≥1 risk factor in the score had scores ≥3.

Performance of the risk score using different cut-points is shown (Supplemental Table 9). The tool identified 33 of 35 (94.3%) infants with invasive HSV with available score calculated who initially presented without a vesicular rash; the 2 infants missed were described in Table 4. When the risk score was applied to the 7 infants with isolated viremia, 3 had missing predictor variables; the HSV risk score for the remaining for infants ranged from 0 to 9. The probability of invasive HSV predicted by the score calibrated well with the observed probability of invasive HSV in all score stratifications (Supplemental Fig 3).

TABLE 2 Multivariable Analyses with the Dependent Variable Being the Presence of Any HSV Infection (ie, SEM or Disseminated)

Candidate Predictor	All Controls, aOR (CI)	Seasonal Controls, aOR (CI)	Random Controls, aOR (CI)	Age-Matched Controls, aOR (CI)
Age				
<14 d	3.3 (1.7 to 6.4)	5.5 (2.6 to 11.9)	5.3 (2.6 to 10.8)	—
14–28 d	2.9 (1.5 to 5.8)	3.7 (1.7 to 8.0)	3.6 (1.7 to 7.8)	—
>28 d	Ref	Ref	Ref	—
Duration of illness				
>1 d	2.2 (1.4 to 3.5)	2.7 (1.4 to 5.1)	—	2.0 (1.2 to 3.4)
≤1 d	Ref	Ref	—	Ref
Abnormal triage temperature[a]	—	—	—	1.8 (1.1 to 3.2)
Irritable	0.4 (0.2 to 0.7)	0.4 (0.2 to 0.9)	0.3 (0.1 to 0.7)	0.5 (0.2 to 0.97)
Had seizure at home	4.4 (2.0 to 9.3)	5.1 (1.8 to 14.2)	6.9 (2.2 to 21.3)	2.9 (1.2 to 6.9)
Ill appearance	3.2 (1.7 to 5.9)	2.4 (1.1 to 5.0)	3.3 (1.4 to 7.5)	3.8 (1.8 to 7.8)
Nonvesicular rash	2.3 (1.2 to 4.6)	3.1 (1.3 to 7.7)	2.5 (1.1 to 5.9)	2.4 (1.1 to 5.3)
Vesicular rash[b]	74.6 (31.3 to 177.6)	133.0 (24.5 to 721.5)	139.1 (26.4 to 732.3)	56.1 (18.1 to 174.0)
Thrombocytopenia[c]	2.8 (1.1 to 6.9)	—	—	2.6 (1.0 to 6.8)
Neutropenia[d]	—	3.3 (1.05 to 10.4)	—	—
CSF pleocytosis[e]	2.6 (1.2 to 5.4)	3.1 (1.4 to 7.0)	3.1 (1.2 to 8.2)	—
Hypoglycorrhachia[f]	—	—	—	2.1 (1.1 to 4.2)

Ref, reference group; —, the covariate was not statistically significant in the regression model.

[a] Triage temperature ≥38.0°C (100.4°F) or <36.4°C (97.5°F).

[b] The ORs and 95% CIs obtained from the penalized logistic regression with 95% previous limits on the OR scale (0.5 to 16) were 46.2 (95% CI: 22.6 to 94.5), 37.1 (95% CI: 15.0 to 92.2), 29.0 (95% CI: 13.1 to 64.0), and 36.9 (95% CI: 14.8 to 91.6) for the regression models including all controls, time controls only, age controls only, and random controls only, respectively.

[c] Platelet count <150 000 per mm^3.

[d] ANC <1000 cells per mm^3.

[e] CSF WBC >15 per mm^3 (≤28 d) or ≥10 per mm^3 (>28 d).

[f] CSF glucose <40 mg/dL.

TABLE 3 Invasive HSV Risk Score

Factor	Point(s)
Age	
<14 d	3
14–28 d	2
>28 d	0
Seizure at home	2
Ill appearance[a]	2
Abnormal triage temperature[b]	1
Vesicular rash	4
Thrombocytopenia[c]	2
CSF pleocytosis[d]	2
Prematurity[f]	1

[a] "Sick, toxic, shocky," altered or decreased mental status, fussy, inconsolable, meningismus (ie, positive Kernig or Brudzinski sign or stiff neck), petechial rash, decreased perfusion, decreased pulses.[11]
[b] Triage temperature ≥38.0°C or <36.4°C.
[c] Platelets <150 000 per mm^3.
[d] CSF WBC count >15 cells per mm^3 if ≤28 d; ≥10 cells per mm^3 if >28 d.
[f] Birth before 37 wk gestation.

DISCUSSION

In our nested case-control study, we derived an invasive HSV risk score that incorporates 8 predictors, including demographics, history, examination, and laboratory tests. Several predictors (ill appearance, pyrexia or hypothermia, and vesicular rash) were associated with invasive HSV infection. Our score accurately identified infants at low risk for invasive HSV infection who may not require HSV testing or empirical acyclovir administration.

Our HSV risk score has face validity and clinical applicability. The selected cut-point optimizes sensitivity, which is crucial given that treatment delays in this vulnerable population are associated with morbidity and mortality.[2] With a negative likelihood ratio of 0.11, and a pretest probability of 0.42%,[5] the posttest probability of disease among those with a low-risk score is 0.05% (ie, 5 per 10 000). We believe our score can reduce HSV testing and treatment when the likelihood of disease is very low. Moreover, lack of access to rapid HSV molecular diagnostic results is associated with prolonged hospitalization[7] and acyclovir therapy; these undesirable outcomes could be mitigated through use of our risk score.

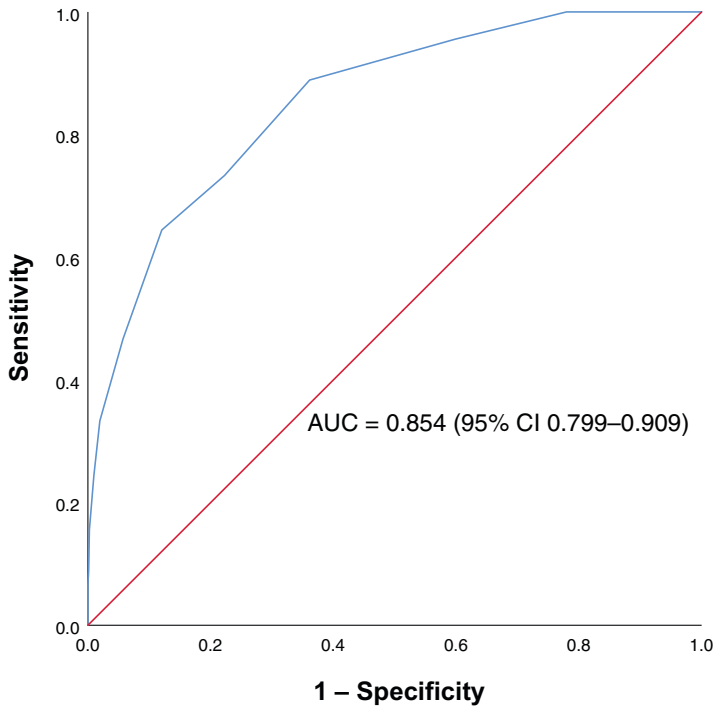

FIGURE 2
Invasive HSV risk score receiver operating characteristic curve. Analysis included 867 (58.2%) infants, including 83 of 149 (55.7%) with HSV infection. The AUC was 0.854 (95% CI: 0.799 to 0.909). When using a cut-point of ≥3, the HSV risk score had a sensitivity of 95.6% (95% CI: 84.9% to 99.5%), specificity of 40.1% (95% CI: 36.8% to 43.6%), and positive likelihood ratio 1.60 (95% CI: 1.5 to 1.7), and negative likelihood ratio 0.11 (95% CI: 0.03 to 0.43).

Our invasive HSV risk score is the first that combines previously recognized predictors[3,4,14–16,22,23] into a risk stratification model. Given that the majority of neonatal HSV cases are caused by peripartum transmission,[24] age is associated with neonatal HSV infection. In previous studies, >80% of cases occurred during the first 21 days of life and nearly 90% occurred by 28 days.[3,22] In our study, most infants with HSV were ≤28 days of age, and age was inversely associated with infection in all models. Although our invasive HSV risk score recommends HSV testing and empirical treatment of most febrile infants ≤28 days of age, because evaluation for HSV infection may be driven by factors other than pyrexia, in such cases our tool can be used to effectively risk stratify. Additionally, in our study, not all infants with invasive HSV infection were tested and had treatment initiated while in the ED; the invasive HSV score may minimize the occurrence of such events. Inclusion of CSF pleocytosis as a high-risk predictor supports the recommendation to empirically treatment these infants for HSV while awaiting test results.[16] With the exception of ill appearance, the remaining predictors are objective and can aid clinicians in identifying infants with invasive HSV.

Other candidate variables associated with HSV infection (eg, elevated CSF RBC counts,[23] transaminitis) that were not included in our final model deserve mention. Although liver transaminases can be elevated in HSV-infected infants, our findings are consistent with a previous study in which only one-third of infants

TABLE 4 Infants with Invasive HSV Infection Misclassified as Low-Risk by the Invasive HSV Risk Score When a Cutoff of ≥3 Is Used

Variable	Case No. 1	Case No. 2
HSV disease classifications	CNS	Disseminated
Age, d	32	16
Prematurity	No	No
Had seizure at home	No	No
Appearance	Well	Well
Temperature at triage, °C	36.9	37.0
Rash	No	No
Thrombocytopenia	No	No
CSF WBC, cells per mm^3	31	7
HSV risk score	2	2
Delivery mode	Vaginal	Vaginal
Maternal genitourinary HSV	No	—
Illness duration, d	>1	<1
Poor feeding	No	Yes
Irritable	No	—
Lethargic	No	—
ALT, U/L	22	58
AST, U/L	46	74
Glucose, mg/dL	95	93
ANC <1000 cells per mm^3	No	No
CSF RBC, cells per mm^3	24	0
CSF glucose, mg/dL	47	38

ANC, absolute neutrophil count; RBC, red blood cell; —, indicates missing.

with disseminated HSV had an elevated ALT.[3] We were unable to include hepatic transaminases and maternal HSV history in our risk score because of excessive amounts of missing data. However, the presence of transaminitis, synthetic hepatic dysfunction, and maternal HSV history[25,26] should raise concerns for HSV infection in the appropriate clinical scenario.

Our study had several limitations. First, historical and clinical features, including maternal HSV history, were frequently missing from the medical record, and laboratory evaluations were not standardized. However, we used multiple imputation methods to avoid the bias inherent in a complete case approach and selected candidate predictors with minimal missing data. However, it is possible that the missing-at-random assumption required by this method does not hold for some of the variables such as maternal lesion (eg, if a response was more likely documented when present). Second, the abstraction of historical and clinical features was potentially subjective; we did not measure interrater reliability of data abstraction. However, we provided a standardized approach and a manual of operations to optimize training and consistency. Third, not all control infants were tested for HSV, but we used short-term clinical follow-up to exclude HSV infection in untested infants. Fourth, we do not know why HSV testing was performed, and the risk of disease likely varies between indications. Fifth, our relatively high proportion of SEM HSV infection in the second month of life may reflect changes in health-seeking behaviors or may be due to changes in viral transmission. Sixth, given the small number of infants with HSV infection in our cohort, CIs were broad. Seventh, although patient eligibility ended several years ago, there is no evidence that the epidemiology of, or risk factors for, invasive HSV has changed that would render our findings less relevant. Finally, although our HSV score should be externally validated before broad implementation, prospective validation will be challenging given the low frequency of HSV infection.

CONCLUSIONS

We identified 8 independent predictors of invasive HSV infection: age, prematurity, seizure before hospital arrival, ill appearance, abnormal triage temperature, vesicular rash, thrombocytopenia, and CSF pleocytosis. These variables, when combined into an invasive HSV risk score, accurately identified infants at extremely low risk for invasive HSV infection and for whom routine HSV testing and treatment can be safely avoided.

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ABBREVIATIONS

ALT: alanine aminotransferase
aOR: adjusted odds ratio
AST: aspartate aminotransferase
AUC: area under the curve
CI: confidence interval
CNS: central nervous system
CSF: cerebrospinal fluid
ED: emergency department
HSV: herpes simplex virus
IBI: invasive bacterial infection
IQR: interquartile range
PCR: polymerase chain reaction
SEM: skin, eyes, and mouth
WBC: white blood cell

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Dr Cruz conceptualized and designed the study, coordinated and supervised data collection, drafted the initial manuscript, and reviewed and revised the manuscript; Drs Nigrovic and Freedman conceptualized and designed the study, assisted with data analysis, and reviewed and revised the manuscript; Dr Xie conducted initial and subsequent analyses, contributed to the writing of the methods and results, and reviewed and revised the manuscript; Drs Mahajan, Thomson, Okada, Uspal, Mistry, Garro, Schnadower, Kulik, Curtis, Miller, Fleming, Lyons, Balamuth, Arms, Louie, Aronson, Thompson, Ishimine, Schmidt, Pruitt, Shah, Grether-Jones, and Bradin performed data collection and regulatory activities at their sites and critically reviewed the manuscript for important intellectual content; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

We derived a risk score based on historical, examination, and laboratory variables that identified 0–60-day-old infants at low risk for invasive HSV infection.

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REFERENCES

1. Flagg EW, Weinstock H. Incidence of neonatal herpes simplex virus infections in the United States, 2006. *Pediatrics*. 2011;127(1):e1–e8
2. Shah SS, Aronson PL, Mohamad Z, Lorch SA. Delayed acyclovir therapy and death among neonates with herpes simplex virus infection. *Pediatrics*. 2011;128(6):1153–1160
3. Curfman AL, Glissmeyer EW, Ahmad FA, et al. Initial presentation of neonatal herpes simplex virus infection. *J Pediatr*. 2016;172:121–126.e1
4. James SH, Kimberlin DW. Neonatal herpes simplex virus infection. *Infect Dis Clin North Am*. 2015;29(3):391–400
5. Cruz AT, Freedman SB, Kulik DM, et al; HSV Study Group of the Pediatric Emergency Medicine Collaborative Research Committee. Herpes simplex virus infection in infants undergoing meningitis evaluation. *Pediatrics*. 2018;141(2):e20171688
6. Brower LH, Wilson PM, Murtagh Kurowski E, et al. Using quality improvement to implement a standardized approach to neonatal herpes simplex virus. *Pediatrics*. 2019;144(2):e20180262
7. Aronson PL, Cruz AT, Freedman SB, et al; Pediatric Emergency Medicine Clinical Research Network (PEM CRC) Herpes Simplex Virus (HSV) Study Group. Association of herpes simplex virus testing with hospital length of stay for infants ≤60 days of age undergoing evaluation for meningitis. *J Hosp Med*. 2019;14(8):492–495
8. Shah SS, Volk J, Mohamad Z, Hodinka RL, Zorc JJ. Herpes simplex virus testing and hospital length of stay in neonates and young infants. *J Pediatr*. 2010;156(5):738–743
9. Downes KJ, Boge CLK, Baro E, et al. Acute kidney injury during treatment with intravenous acyclovir for suspected or confirmed neonatal herpes simplex virus infection. *J Pediatr*. 2020;219:126–132.e2
10. Herpes simplex. In: Kimberlin DW, Brady MT, Jackson MA, eds. *Red Book: 2018 Report of the Committee on Infectious Diseases*, 31st ed. Elk Grove Village, IL: American Academy of Pediatrics; 2018:437
11. Schnadower D, Kuppermann N, Macias CG, et al; American Academy of Pediatrics Pediatric Emergency Medicine Collaborative Research Committee. Febrile infants with urinary tract infections at very low risk for adverse events and bacteremia. *Pediatrics*. 2010;126(6):1074–1083
12. Gilbert EH, Lowenstein SR, Koziol-McLain J, Barta DC, Steiner J. Chart reviews in emergency medicine research: where are the methods? *Ann Emerg Med*. 1996;27(3):305–308
13. Thomson J, Sucharew H, Cruz AT, et al; Pediatric Emergency Medicine Collaborative Research Committee (PEM CRC) HSV Study Group. Cerebrospinal fluid reference values for young infants undergoing lumbar puncture. *Pediatrics*. 2018;141(3):e20173405
14. McGoogan KE, Haafiz AB, González Peralta RP. Herpes simplex virus hepatitis in infants: clinical outcomes and correlates of disease severity. *J Pediatr*. 2011;159(4):608–611
15. Kotzbauer D, Frank G, Dong W, Shore S. Clinical and laboratory characteristics

of disseminated herpes simplex virus infection in neonates. *Hosp Pediatr*. 2014;4(3):167–171

16. Caviness AC, Demmler GJ, Swint JM, Cantor SB. Cost-effectiveness analysis of herpes simplex virus testing and treatment strategies in febrile neonates. *Arch Pediatr Adolesc Med*. 2008;162(7):665–674
17. Bouwmeester W, Zuithoff NPA, Mallett S, et al. Reporting and methods in clinical prediction research: a systematic review. *PLoS Med*. 2012; 9(5):1–12
18. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. *Stat Med*. 2011;30(4):377–399
19. von Hippel PT. How many imputations do you need? A two-stage calculation using a quadratic rule. *Sociol Methods Res*. 2020;49(3):699–718
20. Rubin D. *Multiple Imputation for Nonresponse in Surveys*. New York, NY: Wiley; 1987
21. Herrell F Jr. *Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis*. New York, NY: Springer; 2010
22. Gutierrez KM, Falkovitz Halpern MS, Maldonado Y, Arvin AM. The epidemiology of neonatal herpes simplex virus infections in California from 1985 to 1995. *J Infect Dis*. 1999;180(1): 199–202
23. Caviness AC, Demmler GJ, Selwyn BJ. Clinical and laboratory features of neonatal herpes simplex virus infection: a case-control study. *Pediatr Infect Dis J*. 2008;27(5):425–430
24. Kimberlin DW. Neonatal herpes simplex infection. *Clin Microbiol Rev*. 2004;17(1):1–13
25. Whitley RJ, Nahmias AJ, Visintine AM, Fleming CL, Alford CA. The natural history of herpes simplex virus infection of mother and newborn. *Pediatrics*. 1980;66(4):489–494
26. Brown ZA, Benedetti J, Ashley R, et al. Neonatal herpes simplex virus infection in relation to asymptomatic maternal infection at the time of labor. *N Engl J Med*. 1991;324(18):1247–1252