Plasma and Cerebrospinal Fluid Herpes Simplex Virus Levels at Diagnosis and Outcome of Neonatal Infection

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Objective To evaluate the utility of quantitative herpes simplex virus (HSV) polymerase chain reaction (PCR) levels for prognosis and management of neonatal HSV disease.

Study design Clinical and virologic data were abstracted by medical record review from neonatal HSV cases treated at Seattle Children's Hospital between 1993 and 2012. HSV PCR results from plasma (n = 47), cerebrospinal fluid (n = 56), or both (n = 40) at the time of diagnosis were available from 63 infants; 26 with skin-eye-mouth (SEM), 18 with central nervous system (CNS), and 19 with disseminated (DIS) disease.

Results Plasma HSV PCR was positive in 78% of the infants with SEM, 64% with CNS and 100% with DIS disease. Mean plasma viral level was 2.8 $\log_{10}$ copies/mL in SEM, 2.2 $\log_{10}$ copies/mL in CNS, and 7.2 $\log_{10}$ copies/mL in DIS infants. The HSV levels were higher among infants who died compared with surviving infants, 8.1 $\log_{10}$ copies/mL (range 7.7-8.6) vs 3.8 log10 copies/mL (range 0.0-8.6), $P = .001$, however, level of HSV DNA in the cerebrospinal fluid or in plasma did not correlate with neurologic outcome. Dynamics of HSV clearance from plasma during high-dose acyclovir treatment showed single-phase exponential decay with a median viral half-life of 1.26 days (range: 0.8-1.51).

Conclusions Plasma HSV levels correlate with clinical presentation of neonatal HSV disease and mortality, but not neurologic outcome. *(J Pediatr 2015;166:827-33)*.

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Detection of herpes simplex virus (HSV) DNA by qualitative polymerase chain reaction (PCR) from cerebrospinal fluid (CSF) is used routinely for diagnosis of neonatal HSV central nervous system (CNS) infection,[1-3] and a recent paper reported the use of qualitative HSV PCR from plasma for the diagnosis of neonatal HSV infection.[4] However, little available data exist to inform the use of serial quantitative HSV PCR to evaluate prognosis or therapeutic efficacy during acyclovir (ACV) treatment for neonatal HSV disease. Previous reports on the use of HSV PCR for diagnosis and management of neonatal HSV were either based on samples run retrospectively,[5-8] the cases were selected on the basis of a positive HSV PCR,[4] or the sample size was small.[9] In addition, as the previous reports spanned the time periods prior to the routine use of high-dose ACV for the treatment of neonatal HSV, many infants were not treated[6] or were treated with vidarabine[5] or with low dose and/or short course ACV.[5,7,8]

At Seattle Children's Hospital (SCH), the standard diagnostic work-up for neonatal HSV infection includes viral cultures from mucosal surfaces and lesions, if present, and HSV PCR from plasma and CSF. CSF has been evaluated routinely by HSV PCR starting in 1993, and both CSF and plasma have been tested routinely by quantitative HSV PCR in all infants diagnosed with neonatal HSV since 2001. We report on our experience using quantitative HSV DNA PCR from plasma and CSF for diagnosis and management of neonatal HSV infection and describe the HSV DNA decay rate during high dose ACV therapy.

Methods

Medical records of all infants treated at SCH for virologically confirmed HSV infection during the neonatal period between the years 1993 and 2012 were reviewed. All infants with at least 1 HSV DNA PCR result from either CSF, plasma,

ACV	Acyclovir
ALT	Alanine aminotransferase
CNS	Central nervous system
CSF	Cerebrospinal fluid
DIS	Dissemination
HSV	Herpes simplex virus
PCR	Polymerase chain reaction
SCH	Seattle Children's Hospital
SEM	Skin-eye-mouth

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or both were included. Standardized case report forms were used to extract maternal history of HSV infection (including HSV serology, if available), birth history, clinical presentation and laboratory tests, including coagulation studies, alanine aminotransferase (ALT) and aspartate aminotransferase serum levels, and CSF analysis. Results of all available HSV specific tests, including viral cultures and direct fluorescent antigen obtained from mucosal surfaces and lesions, as well as HSV serology and HSV DNA PCR from plasma and CSF, were recorded. Infants were classified as having skin-eye-mouth (SEM), CNS, or disseminated (DIS) disease according to standard criteria.[5,10] Infants with disseminated infection were classified as DIS whether or not they had concurrent clinical CNS disease.

Long-term outcomes were determined by review of all SCH hospitalizations and clinic visits subsequent to the initial treatment of neonatal HSV infection. Children were classified as normal at longest follow-up if they were meeting age appropriate milestones with no overt evidence of motor impairment and if school age, in age-appropriate classroom. They were classified as mildly impaired if the notes indicated mild visual abnormalities, mild speech delay, or learning disorder without evidence of motor impairment and age-appropriate grade level; moderately impaired if they had spastic diplegia or fine motor delays or had some developmental delay but were able to attend school in special education classes or developmental preschool; and severely impaired if there were severe cognitive abnormalities with microcephaly, spastic quadriplegia, or cortical blindness.

The study was approved by the SCH Institutional Review Board. Records were reviewed retrospectively for all infants born prior to 2011. Infants born after March 2011 were enrolled into a prospective cohort study after informed consent was obtained from parents.

Virologic Evaluation

Serum was tested for HSV antibodies using the University of Washington western blot assay.[11] Western blots were considered positive with full profiles if there were 4 or more HSV type-specific bands.[12] Viral culture was performed as previously described; each isolate was confirmed and typed with monoclonal antibodies.[13,14] HSV DNA was detected with a real-time, quantitative PCR assay that amplified a fragment of glycoprotein B.[15] This assay has been validated extensively; quantities greater or equal to 1 copy per reaction (50 copies/mL) were considered positive.[16] HSV-1 or 2 typing from the PCR product was performed as described previously.[17] Throughout this manuscript, HSV level is used to indicate HSV DNA concentration (copies/mL) by PCR.

Infants were considered to lack type-specific antibody at diagnosis if testing on serum drawn from the infant or the mother at diagnosis was negative by western blot for antibodies to the viral type with which the infant was infected. Infants were considered to have type-specific antibody at diagnosis only if testing from the infant was positive by western blot for antibodies to the viral type with which the infant was infected. Infants without antibody results at diagnosis and whose mothers had detectable antibodies to the infecting viral type were excluded from this part of the analysis as timing of maternal infection could not be determined. One additional infant with detectable antibodies to the infecting viral type was excluded because he had received multiple transfusions including fresh frozen plasma, potentially containing HSV antibodies.

Statistical Analyses

The χ^2 and Fisher exact tests were used to examine differences in categorical characteristics by infection status. Linear regression was used to estimate differences by infection status in continuous measures including quantity of virus in plasma and CSF. Logistic regression was used to examine predictors (clinical CNS disease, CSF PCR) of neurologic outcome. Because of varying length of follow-up and the tendency for healthy children to end follow-up earlier than children with more severe diagnoses, comparisons involving neurologic outcomes were performed using detection within 2 months. Two-sided P values of $\leq .05$ were considered statistically significant. Risk of death in infants using quantitative predictors log10(ALT) and log10(HSV PCR copy number) was computed using Poisson regression.

To capture HSV plasma dynamics, DEDiscover (http://cbim.urmc.rochester.edu/) was used to fit an exponential decay model to each individual curve. The model has a single unknown variable, the clearance rate of virus in plasma.

Results

Infants with neonatal HSV infection (n = 63) had HSV PCR results from plasma (n = 47), CSF (n = 56), or both (n = 40) at the time of diagnosis; 26 infants (41%) had SEM disease, 18 (29%) had CNS, and 19 (30%) had DIS disease (**Table I**). Infants presenting with CNS disease were older (mean age at diagnosis 17 days) than infants with SEM (mean age 9 days, $P < .001$) and DIS (mean age 7 days, $P < .001$) disease. More infants with CNS (15 of 18) vs SEM (7 of 26) disease were infected with HSV-2 ($P = .001$). Six of 19 (32%) with DIS disease died in the neonatal period, all within 3 weeks of birth. Overall, there was no difference in mortality between infants infected with HSV-1 vs HSV-2. Four of the 13 surviving infants with DIS also had CNS disease based on CSF pleocytosis at presentation. Of the 16 infants with DIS with antibody results available at diagnosis, none had HSV antibodies against their infecting viral type, consistent with maternal acquisition of primary HSV infection late in pregnancy. In contrast, most infants presenting with CNS disease (11 of 15, 73%) had HSV antibodies against their infecting viral type at presentation ($P < .001$).

All infants received treatment with ACV at 60 mg/kg/d[18] except for one with CNS disease and 4 with SEM disease treated with 45 mg/kg/d. All infants with CNS and DIS disease, or with detectable HSV in the CSF regardless of clinical presentation, received at least 21 days of ACV. Five of the 12 infants with SEM with positive plasma HSV PCR received