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Plasma and CSF herpes simplex virus levels at diagnosis and outcome of neonatal infection

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Abstract

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 (CSF) (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 log₁₀ copies/mL in SEM, 2.2 log₁₀ copies/mL in CNS, and 7.2 log₁₀ copies/mL in DIS infants. The HSV levels were higher among infants who died compared with surviving infants, 8.1 log₁₀ copies/mL (range 7.7–8.6) vs 3.8 log₁₀ copies/mL (range 0.0–8.6), p=0.001, however, level of HSV DNA in the CSF 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).

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Conclusion—Plasma HSV levels correlate with clinical presentation of neonatal HSV disease and mortality, but not neurologic outcome.

Keywords

HSV; PCR; disseminated; neonates

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 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 one HSV DNA PCR result from either CSF, plasma 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 (AST) 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), central nervous system (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 pre-school; 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 polymerase chain reaction 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

Chi-square 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 followup 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 two months. Two-sided p-values ≤ 0.05 were considered statistically significant. Risk of death in infants using quantitative predictors $\log_{10}(\text{ALT})$ and $\log_{10}(\text{HSV PCR copy number})$ 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, $p<0.001$) and DIS (mean age 7 days, $p<0.001$) disease. More infants with CNS (15 of 18) vs SEM (7 of 26) disease were infected with HSV-2 ($p=0.001$). 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 DIS infants also had CNS disease based on CSF pleocytosis at presentation. Of the 16 DIS infants 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 ($p<0.001$).

All infants received treatment with ACV at 60mg/kg/day [18] except for one with CNS disease and 4 with SEM disease treated with 45 mg/kg/day. 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 SEM infants with positive plasma HSV PCR received 14 days of ACV; the remainder were treated with at least 21 days. Eight infants (3 SEM, 3 CNS, 2 DIS) were placed given oral suppressive acyclovir after their initial intravenous treatment. One of these infants was enrolled in a clinical trial evaluating suppressive acyclovir therapy [19].

HSV DNA PCR and diagnosis of neonatal HSV

Of the infants with plasma results available, HSV DNA was detectable in all with DIS and in most with SEM and CNS disease (Table II). CSF samples were positive by PCR at diagnosis in 13 of the 18 infants with clinically diagnosed CNS disease and 3 of the 4 DIS infants with clinical evidence of concurrent CNS disease. HSV DNA was also detected by PCR at diagnosis in the CSF of 2 of the 24 infants with SEM disease (both infants had blood contamination of the CSF) and 4 of the 7 DIS infants without clinical evidence of concurrent CNS disease who had CSF tested at diagnosis. Cultures of surface specimens grew HSV from at least one mucocutaneous site from 96% of the infants diagnosed with SEM disease, but only from half of the infants with CNS disease. Although no single diagnostic test (plasma PCR, CSF PCR, surface cultures) was positive for all infants, plasma PCR was the most frequently positive test with 83% of infants having a positive plasma PCR. In the infants with plasma HSV PCR available, the plasma HSV PCR was the only positive result for 5 of 47(11%): 2/18 SEM, 1/11 CNS, 2/18 DIS.

Forty-one infants had quantitative HSV plasma PCR results from the time of initial diagnosis (Figure 1, A). Mean HSV level in plasma was similar in infants with SEM and

CNS disease (2.8 log₁₀ copies/ml vs 2.2 log₁₀ copies/ml, difference, -0.6, 95% CI -2.0-0.9, p=0.45), but the mean HSV level was significantly higher in DIS infants (7.2 log₁₀ copies/ml, difference 4.5, 95% CI 3.2-5.8, p<0.001) compared with infants with SEM or CNS disease. HSV levels and serum ALT levels were correlated ($\rho = 0.59$) and both were associated with the risk of death. However, in a model that included both ALT and HSV levels, only HSV level (log₁₀ copies/ml) remained associated with death, with the risk of death 4.1 times higher for each 1 unit increase in log₁₀ copies/ml (95% CI 2.2 to 7.7; p<0.0001). Of interest, no infant with a plasma HSV viral level ≥ 7 log₁₀ copies/ml died in the neonatal period. No difference in the plasma HSV levels between infants infected with HSV-1 vs HSV-2 was observed, (4.4 vs 4.3, p=0.93). Fifty infants had quantitative HSV PCR results from CSF at the time of initial diagnosis (Figure 1, B). Compared with infants with SEM, mean quantity of HSV detected in CSF (0.1 log₁₀ copies/ml SEM) was higher in those with CNS disease (2.1, difference = 2.0, 95% CI 1.2-2.9, p<0.001) and in those with DIS disease (1.8, difference = 1.7, 95% CI 0.8-2.6, p=0.001).

CSF HSV DNA levels and neurologic outcome

Neurologic outcome could be assessed in all but 6 children who died and 8 who had no follow up after hospital discharge from their initial treatment. Of the 49 infants alive with at least 2 months of follow-up, 8 were quickly diagnosed with neurologic deficits: median time from diagnosis of neonatal HSV to diagnosis of deficit was 17 days (range 0-57). All 8 infants had CNS disease and were confirmed as having severe impairment in 6 (75%) and moderate impairment in 2 (25%) at longest follow-up. All children with SEM disease were normal at longest follow-up, though SEM children were somewhat less likely to have at least 2 months of follow-up (Table I). Mild to moderate neurologic deficits were noted more than 2 months after diagnosis in an additional 3 children with CNS and in 2 with DIS. One DIS child had speech and fine motor delays and autistic behaviors at 5 years of age. This infant had a negative CSF PCR at diagnosis and no clinical evidence of CNS disease. Another DIS child had mild cognitive delay at 5 years of age with normal motor skills. This child had a positive CSF PCR and pleocytosis at diagnosis. Both of these infants had multiple skin recurrences documented in the first 6 months of life.

Among those who survived, mean CSF HSV PCR DNA level at presentation did not differ statistically between the infants who were normal at 2 months (1.0 log₁₀ copies/ml; range 0-4.6) vs those with early neurologic abnormality (1.9 log₁₀ copies/ml; range 0-5.5) p=0.19. Of note, all 6 infants with HSV DNA detected in the CSF by PCR but without clinical evidence of CNS disease at the time of initial illness were normal at longest follow-up (median follow-up 21 months; range 10 months-11 yrs), whereas only 3 of the 6 infants with clinical evidence of CNS disease but without HSV DNA detected in the CSF by PCR at diagnosis were normal at longest follow-up. Thus the presence of clinical CNS disease at diagnosis was a stronger predictor of neurologic outcome than was the presence of HSV DNA in the CSF.

Dynamics of HSV viral clearance during treatment for disseminated disease

Six of the 19 infants with DIS disease had sufficient serial quantitative plasma HSV DNA measurements for fit to a viral decay model to determine the dynamics of HSV clearance

during high-dose ACV treatment. All had severe hepatitis (ALT >10× normal) and 5 also had disseminated intravascular coagulopathy and pneumonia. Two were infected with HSV-1 and 4 with HSV-2. A single-phase exponential decay model ($dV/dt = -(\delta \cdot V)$) reproduced primary clearance kinetics (median $R^2 = 0.96$, range 0.91–0.99) in these infants. Primary clearance continued until extinction in 5 infants (Figure 2). In one infant a 21-day primary clearance phase was followed by persistent low-level viremia lasting 75 days prior to extinction (Figure 2). Median viral half-life was 1.26 days (range: 0.8 – 1.51 days) during primary phase of decay in the six infants.

Discussion

In our large cohort of infants with virologically confirmed neonatal herpes, we found a clear association between the plasma HSV level, clinical presentation and outcome, with death confined to children with very high plasma HSV levels at presentation. In contrast, clinical evidence of CNS disease, rather than CNS HSV level, predicted neurologic outcome. Decay of HSV in plasma in treated infants showed a single-phase exponential decay.

In the DIS infants with serial viral levels, ACV had a uniform and highly predictable effect on plasma HSV regardless of baseline viral level at the initiation of therapy. The muted immunologic response in infants with systemic HSV infection [20] is highlighted by the long plasma half-life of HSV DNA (1 day) compared with the 2-hour half-life of HSV DNA in adult recurrent genital lesions [21] where an intense cellular immune response accompanies viral replication [22–24]. One infant demonstrated low level plateau viremia for many weeks after a robust primary clearance phase; the reason for this is unclear but could represent ongoing HSV replication within drug sanctuaries from drug exposure. Acyclovir treatment was continued in infants who remained HSV plasma positive at the end of the standard 21-day treatment in most cases until the plasma PCR was negative. Our data are not conclusive regarding the risk of discontinuing ACV prior to complete clearance of plasma virus. However, in the context of clinical worsening, an increase in the plasma HSV DNA level may be an indication for restarting or changing antiviral treatment or questioning the dosing adequacy, as in the case of infants receiving extracorporeal membrane oxygenation (ECMO) where we have little to no guidance on dosing for acyclovir. Our findings of very high viral levels with slow decay in infants with disseminated HSV disease are consistent with a lack of immune protection in neonates born to women who acquired HSV late in pregnancy and an immature immune response. Alternatively, or additionally, infants with disseminated HSV may have received a higher inoculum of virus from their mothers, as women with primary infection shed large amounts of virus in genital secretions [25, 26].

Most infants with SEM disease had detectable HSV DNA in their plasma. There was no specified protocol for treatment of SEM disease with viremia during the time of this study, and some infants received 21 days of ACV and others only 14 days. All infants with SEM disease cleared their viremia prior to ACV discontinuation and were neurologically normal at longest follow-up regardless of length of ACV therapy; as such the need for longer treatment in infants with HSV viremia but without clinically disseminated disease is unclear. That the positive plasma PCR represents viremia rather than just circulating viral particles is

supported by previous reports of blood cultures positive for HSV in infants with neonatal disease [27] and demonstration in adults with primary genital herpes that HSV recovered from plasma is encapsulated within virions [28]. Nevertheless, our data suggest that in the absence of any evidence of organ involvement, detectable HSV DNA in plasma by PCR may not by itself denote requirement for prolonged intravenous ACV therapy.

Our results are consistent with an earlier study conducted retrospectively by Kimura et al using stored serum samples [8]. Similarly, they showed that infants who died from disseminated neonatal HSV had very high serum viral levels. In contrast, a study of plasma HSV levels in hospitalized adults with disseminated HSV disease did not find a correlation between viral level and mortality although the number of patients was small (n=13) and cross-sectional sampling may have missed peak viral levels [29].

Our data support the need to utilize multiple diagnostic modalities to confirm or exclude HSV disease in neonates as recommended by the American Academy of Pediatrics Committee on Infectious Diseases [30]. Although plasma HSV PCR was the most frequently positive test, almost half of the infants with CNS disease and one-quarter of infants with SEM disease had negative plasma PCR. Similarly, even though surface cultures were positive in almost all of the infants with SEM, they were less often positive in infants with CNS and DIS. The lower sensitivity of all diagnostic tests for CNS disease highlights the risk of missing the diagnosis of neonatal HSV in these infants and emphasizes the need for clinicians to have a high suspicion for HSV in ill neonates [31]. It is likely that HSV PCR testing from swabs obtained from mucosal surfaces would increase the diagnostic yield compared with viral culture, as has been shown for genital lesions in adults, [32] but few infants in our sample had PCR assays performed on collected surface swabs.

Similar to other reports [5, 6, 8, 33], the sensitivity of HSV DNA detection by PCR in the CSF of neonates with CNS disease in our cohort was around 70%. This is consistent with the major mode of pathogenesis of neonatal HSV CNS disease, which is thought to be neuronal transport to the brain resulting in an initially localized encephalitis prior to involvement of the meninges [31]. Thus early sampling of the CSF may not detect HSV. Conversely, in our cohort, two infants with clinical SEM disease and 4 of the infants with DIS who otherwise had no clinical evidence for CNS disease had HSV DNA in their CSF. In the two infants with SEM, the CSF was contaminated with peripheral blood thus the positive results could have been from the plasma. Similarly, it is not clear if the detectable HSV DNA in the CSF of the 4 infants with DIS represents true CNS infection, or if the extremely high plasma viral levels ($> 7 \log_{10}$ copies/ml) could have allowed crossover into the CSF. Although this observation would not change the recommendation for at least 21 days of high-dose ACV treatment for any infant with detectable HSV DNA in the CSF, it does suggest that infants with clinical DIS and SEM disease but without clinical evidence of concurrent CNS disease are likely to have a good neurologic prognosis, if they survive the early infection. The lack of correlation between CSF HSV DNA levels and neurologic outcome may reflect an incomplete association between tissue damage and DNA concentration in the CSF or that indirect causes of tissue damage such as inflammation contribute to CNS pathology [34].

We categorized maternal infection based on serologic results from infant or mother obtained at the time of the diagnosis. The presence of type-specific antibody appeared to be protective against DIS but not CNS disease. Maternal primary infection late in pregnancy could be assumed in the cases where the infant and/or maternal serology was negative for antibodies to the infecting viral type. This was the situation for all of the infants with DIS and one-half of the infants with SEM. In contrast, most infants with CNS disease had antibodies to their infecting viral type consistent with transplacental passage of maternal antibodies. The greater likelihood of detection of HSV antibodies at the time of diagnosis for infants with CNS disease has been noted in previous studies [35–37]. An alternate explanation is that these infants are infected shortly after birth and have mounted their own antibody response by the time they become symptomatic with HSV encephalitis [38]. This is less likely, as the time to development of a full profile of HSV antibodies after primary infection in adults is approximately 27 days [39].

Because our results are derived from a retrospective review of clinical data, not every infant had all of the recommended testing and repeat virologic testing was not done systematically. In addition, the assessment of neurologic outcome was based on chart notes from clinic visits. Given the span of years, the length of follow-up was variable, and it is possible that some of the infants who appeared to be neurologically and developmentally normal at 1–2 years may demonstrate cognitive, behavior or motor problems when they reach school age. Nevertheless, most of the infants in our cohort had an extended period of follow-up and we are confident that any significant neurologic morbidity was detected.

In conclusion, we found that HSV levels in plasma are highly correlated with clinical presentation and mortality, but not neurologic outcome. Our data support the inclusion of plasma PCR in the diagnostic work-up for neonatal HSV infection and suggest that quantitative HSV plasma PCR may provide additional data to guide management of neonatal HSV infection. Further prospective studies are needed to optimize the use of these assays in the care of infants with HSV disease.

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Abbreviations

ACV	acyclovir
ALT	alanine aminotransferase
AST	aspartame aminotransferase
CNS	central nervous system
CSF	cerebrospinal fluid

DNA	deoxyribonucleic acid
DIS	disseminated
ECMO	extracorporeal membrane oxygenation
HSV	herpes simplex virus
HIV	human immunodeficiency virus
PCR	polymerase chain reaction
SCH	Seattle Children's Hospital
SEM	skin, eye, mouth
ULN	upper limit of normal

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Figure 1.

Plasma (1a) and CSF (1b) HSV viral levels at diagnosis according to clinical classification and viral type. Values below the limit of detection (dotted line) were separated for visual clarity. Horizontal bars indicate the median HSV DNA level in copies/mL.

Figure 2. HSV viral clearance in six infants with DIS disease during acyclovir treatment

Black horizontal lines represent duration of IV ACV treatment: in infant 3, acyclovir was stopped prior to clearance of HSV viremia. Viral levels continued to decrease and became undetectable after discontinuation of acyclovir. Infant 6 experienced an increase in HSV DNA copy number shortly after the first discontinuation of acyclovir. This was associated with an increase in serum hepatic transaminases, which prompted retreatment. No acyclovir resistance mutations were detected and the viremia eventually cleared after discontinuation of a second course of acyclovir. This infant was infected with HSV2 and also was positive for CSF PCR. He was neurologically normal at age 5 years. Dashed lines are fit on a simple exponential decay model: for infant 6, only data during the primary decay phase (through day 28) were modeled.

Table 1

Neonatal HSV patient characteristics

Characteristic	SEM n=26	CNS n=18	DIS n=19	Total n=63	P value
Age at diagnosis, median days (range)	9 (2–19)	17 (5–34)	7 (4–15)	9 (2–34)	<0.001
Male sex, # (%)	15 (58)	8 (44)	10 (53)	33 (52)	0.69
Race, # (%) [*]					
White	18 (95)	14 (78)	10 (56)	42 (76)	
Other	1 (5)	4 (22)	8 (44)	13 (24)	0.019
Viral type, # (%)					
HSV-1	19 (73)	3 (17)	8 (42)	30 (48)	
HSV-2	7 (27)	15 (83)	11 (58)	33 (52)	0.001
HSV type-specific antibody present at diagnosis, # (%)					
if HSV-1 type	7/14 (50)	1/2 (50)	0/8 (0)	8/24 (33)	0.032
if HSV-2 type	2/5 (50)	10/13 (77)	0/8 (0)	12/26 (46)	0.001
Death in neonatal period, # (%)	0 (0)	0 (0)	6 (32)	6 (10)	0.001
Age at death, median days(range)	NA	NA	10 (8–18)	10 (8–18)	NA
Length of follow-up, median years(range)	1 (0–11)	4 (0–18)	2 (1–5)	3 (0–18)	0.02
[‡] Followed for at least 2 months to assess deficit	20/26 (77%)	17/18 (95%)	12/13 (92%)	49/57 (86%)	0.29
Neurologic deficit by 2 months [‡]	0/20 (0%)	8/17 (47%)	0/12 (0%)	8/49 (16%)	<0.001
Neurological deficit during follow-up [‡]	0/20 (0%)	11/17 (65%)	2/12 (17%)	13/49 (26%)	<0.001

SEM= skin, eye, mouth disease

CNS=central nervous system disease

DIS=Disseminated disease +/- CNS disease

^{*} race is missing for 13% (n=8) of infants

P-value is either for chi-square test (if categorical) or Fisher exact test (if categorical and low frequencies) or overall F-test from linear regression (for continuous measures).

[‡] Among those 49 who remained alive and had at least 2 months of follow-up.

[‡] Among those 63 who remained alive.

Table 2

Sensitivity of HSV DNA PCR and surface cultures for neonatal HSV syndromes according to sampling site.

	SEM N=26	CNS N=18	DIS N=19	Total N=63	p-value Fisher's test
HSV plasma PCR positive, n (%)	14/18 (78)	7/11 (64)	18/18 (100)	39/47 (83)	0.030
HSV CSF PCR positive, n(%)	2/24 (8)	13/18 (72)	9/14 (64)	24/56 (43)	<0.001
Surface culture positive, n (%)	24 [*] /25 (96)	9 [*] /18 (50)	12/18 (67)	45/61 (74)	0.002

SEM= skin, eye, mouth disease

CNS=central nervous system disease

DIS=Disseminated disease

CSF= cerebrospinal fluid

* two infants (one SEM and one CNS) had pooled nasopharyngeal/conjunctival cultures which were HSV culture negative, but they had skin lesions which were not cultured. These infants were considered surface culture negative in the analysis