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EDITORIAL

Polymerase chain reaction in neonatal HSV encephalitis: an assay to count on?

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Despite advances in clinical management, including the introduction of high-dose acyclovir as a standard pharmacologic therapy, neonatal herpes simplex virus (HSV) infections continue to cause significant mortality and long-term childhood morbidity. Herpes simplex virus central nervous system (CNS) disease, presenting as an isolated encephalitis,[1] as a component of disseminated HSV,[1,2] or as a potential complication of primary[1–3] or recurrent[4] mucocutaneous HSV, contributes significantly to this burden and remains a diagnostic and management challenge. Although prior and current investigations have resulted in decreased mortality in infants with HSV CNS disease (particularly when receiving high-dose acyclovir[5]), we have seen no improvement in the rate of neurologic complications (relative to the pre-antiviral era) in infants with HSV encephalitis.[5–7] Furthermore, the known properties of latency and replication of HSV in neurons and the potential for reactivation complicate attempts to understand the evolution and progression of the disease.

In this issue of the *Journal of Perinatology*, Mejias, *et al*.,[8] highlight these issues in a case series of four infants with significant neurological sequelae due to herpes simplex virus infection. Each infant presented initially with abnormal neurologic and radiographic findings consistent with central nervous system (CNS) injury, and was diagnosed with HSV disease by polymerase chain reaction (PCR) assay of cerebrospinal fluid (CSF). Repeat PCRs in all the four cases were positive during or at the end of initial high-dose acyclovir therapy, resulting in an extension of acyclovir therapy beyond the recommended 21-day course for encephalitis[9] until negative results were documented. Despite these interventions, three of the infants developed long-term neurodevelopmental abnormalities in childhood; the fourth infant had persistent seizures before his death at 1 month of age.

The utility of the HSV PCR assay in each of these cases cannot be understated. Initial viral cultures taken in three of the infants were negative, with the PCR results making the final diagnosis in all four neonates. Furthermore, the persistence of PCR products in the CSF led to an extension of antiviral therapy (as has been recommended previously[10]), which likely prevented more significant CNS injury in the surviving three infants. Routine HSV PCR analysis of the CSF should be included in the evaluation of any neonate presenting with signs or symptoms of sepsis. Furthermore, routine CSF PCR analysis during acyclovir therapy, in addition to the standard 'end of treatment' lumbar puncture[10] for infants with HSV encephalitis, may help researchers and clinicians understand the progression of neonatal HSV encephalitis and its correlation to neurodegeneration and long-term abnormal neurodevelopment.

Polymerase chain reaction has long been documented as useful in the evaluation of neonatal HSV disease;[3,11–13] however, problems with the reliability of this technique in its current state may limit its utility in future investigations. The lack of inter-laboratory standards for HSV PCR[1,14] and the use of single-lab-developed assays (termed 'home-brew' protocols by one major textbook[1]) raises the possibility that the same sample tested in different laboratories may provide different results, leading to inappropriate management strategies. The authors of this paper report that the confirmatory PCR for each of the three infants treated in their home institution (a major US academic medical center) was carried out by a different laboratory (one internal, two external). As the diagnosis of the HSV disease in each infant was carried out by PCR alone, a false negative in either case could have led to more significant morbidity and/or mortality due to failure to initiate therapy or early treatment cessation. The authors do not explain whether the use of an outside laboratory for two of the three assays was due to problems with reliability of their in-house assay. However, if PCR is to achieve 'gold standard' status as a diagnostic test in the HSV disease, a uniform PCR assay (or uniform inter-laboratory standards for HSV PCR) must be developed, such that a sample sent to a laboratory in Dallas, Santiago, or Baltimore gives, similar assay results.

Additionally, the sensitivity and specificity of PCR in the current age of neonatal HSV disease must be addressed. Original studies used to generate the recognized utility (sensitivity 75 to 100%, specificity 71 to 100%[1,2]) of HSV PCR in neonatal HSV CNS disease were done on small groups of patients[11–13] or on retrospective analysis of stored CSF from a large NIAID (National Institute of Allergy and Infectious Diseases) study,[3] in which $<20\%$ of the CSF samples assayed were obtained before antiviral therapy. These limitations, as well as differences in design among the studies and complications in specimen preservation, have been previously discussed.[15,16] However, given that at least one-third of the estimated 1500 annual cases of neonatal HSV in the United States result in isolated encephalitis,[1,16,17] a multicenter evaluation of PCR in neonatal HSV CNS disease should be undertaken in hopes

of achieving high sensitivity and specificity (98 and 94%, respectively) of the assay documented in adult HSV encephalitis.[18]

Finally, further investigation of PCR applications in neonatal HSV should also extend beyond the documentation of a single positive assay during the initial evaluation. In this paper, Mejias *et al.* discuss the unknown significance and possible etiologies of a persistently positive HSV PCR after appropriate therapy (including a high viral CSF load, an acyclovir-resistant HSV isolate, or a genetically determined immune deficiency in the host.[8]). Larger studies of HSV encephalitis have shown a decline in HSV DNA detected during the course of antiviral therapy[2,18–20] However, persistently positive PCR results (and poor neurologic outcomes) have been documented in studies of neonatal HSV encephalitis, using lower doses or shorter courses of acyclovir rather than the current standard.[3,11] Although presumed to reflect persistent viral replication, it is unclear whether the detected PCR products after therapy represent active infection, residual DNA fragments after successful treatment, or a point along a continuum between the two states. Quantitative real-time PCR, which can provide rapid detection of HSV DNA and documentation of viral load,[21] has been used successfully in pediatric[22,23] and neonatal[22–24] patients with known HSV CNS infections. Kimura *et al.*[24] applied quantitative real-time PCR in a small population of neonates with HSV, showing an association between HSV-2 infection and higher neurological morbidity, CNS involvement and CSF viral load. Quantitative PCR may be useful in determining the efficacy of antiviral therapy in neonatal HSV encephalitis by documenting HSV DNA levels in serial CSF samples from infected infants. Additionally, in correlation with culture and immunologic studies, the quantitative assay may answer questions about viral resistance, specific neonatal immunodeficiencies and long-term neurodevelopmental outcomes raised by the authors.[8] More importantly, using quantitative HSV PCR to monitor viral load during neonatal HSV encephalitis, and in possible recurrences during childhood, may allow determination of clinical and genetic risk factors for infection (as suggested previously[22]).

The call for continued clinical vigilance by pediatricians for signs and symptoms of neonatal HSV infection has been sounded previously.[17] However, to accomplish this goal and to improve childhood outcomes, clinicians also need a standardized laboratory technique to document and understand the evolution and progression of HSV CNS infection. With the application of a reliable, useful PCR assay, pediatricians can extend the current state of neonatal HSV management to reduce the neurological morbidities that have persisted in the age of antiviral therapy.

Conclusions

Serial analysis of CSF by the PCR may assist in monitoring the progression of neonatal HSV encephalitis. However, PCR is not yet recognized as the optimal diagnostic test in neonatal HSV CNS disease. A uniform, validated HSV PCR assay providing quantitative data is necessary to further study the pathogenesis of herpes infections in newborns, particularly for evaluation of the evolution and outcomes of HSV CNS infections.

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