Diagnosis of Neurosyphilis in Patients Infected with Human Immunodeficiency Virus Type 1

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Establishing the diagnosis of neurosyphilis may be particularly difficult in human immunodeficiency virus type 1 (HIV)-infected persons. Polymerase chain reaction (PCR) was used to detect Treponema pallidum DNA in cerebrospinal fluid (CSF) from 81 HIV-infected patients. On the basis of reactive serum and CSF-VDRL tests, 2 patients were diagnosed as having neurosyphilis. T. pallidum DNA was not consistently detected in any sample, even when the CSF-VDRL was reactive. CSF pleocytosis, elevated protein, or depressed glucose concentration was not significantly associated with a history of exposure to or infection with T. pallidum. On the basis of results of routine CSF measurements and T. pallidum PCR results, no evidence was found for undiagnosed neurosyphilis in HIV-infected patients. T. pallidum DNA PCR on CSF did not provide more information than conventional CSF analysis. Further study is needed to determine the utility of this test in the diagnosis and treatment of neurosyphilis.

Neurosyphilis is a diagnostic challenge in persons infected with human immunodeficiency virus type 1 (HIV). The diagnosis of neurosyphilis is based on clinical findings and identification of cerebrospinal fluid (CSF) abnormalities, such as mononuclear pleocytosis and moderately elevated protein concentration. Reactivity of the CSF-VDRL test confirms the diagnosis, but a nonreactive CSF-VDRL does not exclude it [1]. Because HIV infection alone may be associated with neurologic abnormalities or with CSF pleocytosis and elevated CSF protein concentration [2], establishing the diagnosis of neurosyphilis may be particularly difficult in HIV-infected persons. Identification of Treponema pallidum DNA by polymerase chain reaction (PCR) in CSF samples is an alternative method for confirming central nervous system (CNS) T. pallidum infection that should not be confounded by concomitant HIV infection. The PCR method is sensitive and specific for detection of T. pallidum in CSF [3], and T. pallidum DNA has been detected in CSF from patients with early and late syphilis [3–7]. However, the utility of this test in the diagnosis of neurosyphilis has not been established. We examined the role of conventional CSF analysis and of T. pallidum PCR in diagnosing neurosyphilis in HIV-infected persons.

Methods

Study population. From 1 January 1989 through 30 June 1991, CSF samples from 100 HIV-infected patients undergoing lumbar puncture as part of their clinical care were saved by the Clinical Laboratory at San Francisco General Hospital. In most instances, these samples were stored at −20°C, thawed within 3 weeks of collection, divided into aliquots, and refrozen at −70°C; a few samples were transported directly to the long-term storage laboratory, aliquoted, and frozen at −70°C. All samples, including those for PCR analysis, were processed in facilities free of Treponema pallidum or T. pallidum DNA. Samples from 82 patients were available for PCR analysis. Clinical data, including HIV-related diagnoses, peripheral blood CD4 lymphocyte count, history of and treatment for syphilis, and results of CSF studies were abstracted from patients’ medical records.

Serum and CSF measures. Syphilis serology included serum VDRL, CSF-VDRL, and microhemagglutination tests for T. pallidum [8]. CSF cell counts, protein and glucose concentrations, cryptococcal antigen reactivity, and bacterial, fungal, and viral cultures were performed according to standard clinical laboratory procedures.

PCR. Before PCR amplification, 2 × 10^4 K562 cells (a source of human β globin gene) were added to 0.2- to 0.3-mL CSF samples. The samples were digested with proteinase K, the DNA was precipitated with ammonium acetate-ethanol, and the pellet was air-dried. The pellet was resuspended in DNA purification matrix (InstaGene; BioRad, Hercules, CA), incubated at 56°C for 30 min, boiled for 8 min, and pelleted. Supernatant (50 μL) was amplified in a total volume of 100 μL of reaction mixture using T. pallidum primers 47-1 and 47-2, according to published methods [3]. All specimens were
prepared under a nonventilated enclosure in a dedicated PCR facility. Positive controls included 10–50 T. pallidum added to normal human CSF. Negative controls included reagent blanks and K562 cells added to normal human CSF. To exclude inhibitors, the efficiency of amplification was assessed by amplification of the β globin gene in each specimen [9]. PCR products were examined by dot-blot analysis using biotin-labeled probes 47-3 and 47-4 [3]. Positive samples were retested on a separate CSF aliquot. Samples were not considered to contain T. pallidum DNA unless both PCR reactions were positive. In mock specimens, the limit of detection of the PCR assay was 10–20 T. pallidum in 0.3 mL of normal human CSF. Freezing and thawing the positive control samples twice did not change the limit of detection.

Statistical analyses. Correlations between discrete variables were assessed by χ² or Fisher’s exact test. Associations between risk factors and outcomes were described using odds ratios (OR) with 95% confidence intervals (CI). Confounders were adjusted for by logistic regression. All tests were two-tailed, and P ≤ .05 was considered significant. No adjustments were made for multiple comparisons.

Results

T. pallidum DNA was detected by PCR in 6 of 82 CSF samples on initial analysis. Additional samples were available for 5 of these 6, and repeat PCR tests were negative. A duplicate sample for 1 patient was not available, and this patient was not included in further analyses. Thus, 0 of 81 (CI, 0–3.7%) CSF samples contained T. pallidum DNA by PCR.

Of 81 patients, 41 (51%) had been exposed to or diagnosed as having syphilis, with the first such episode occurring a median of 10.3 years (range, 18 days to 27.3 years) before the lumbar puncture. Prior syphilis therapy was verified for 38 patients. Most patients in this series were significantly immunosuppressed, with median CD4 cell counts of 80/μL (range, 0–920). Twenty-three patients (28%) had received one or more neurologic diagnoses before their lumbar puncture, including 11 with cryptococcal meningitis, 8 with dementia, 4 with CNS toxoplasmosis, and 1 with progressive multifocal leukoencephalopathy. Four patients, 2 with cryptococcal meningitis and 2 with neurosyphilis, were diagnosed as having these diseases because of the lumbar puncture results. The 2 patients with newly defined neurosyphilis had reactive CSF-VDRL and serum VDRL tests. One had a prior history of syphilis, and 1 did not.

We reasoned that occult neurosyphilis, if it occurred, might represent reactivation of previously acquired disease and would therefore be more likely in persons with a history of syphilis. We used linear regression to examine the influence of a history of syphilis or exposure to syphilis on CSF abnormalities, defined as CSF white blood cell count >10/μL (n = 16), CSF protein concentration >45 mg/dL (n = 34), or CSF glucose concentration <40 mg/dL (n = 9). None of these abnormalities was significantly associated with a history of syphilis or exposure to syphilis (OR, 1.2–1.4; P = not significant; data not shown). Controlling for known current or past neurologic disease and for CD4 cell count <200/μL (which influences development of neurologic disease) did not strengthen the associations (data not shown).

Discussion

Our data suggest that T. pallidum DNA is not commonly detected in CSF of HIV-infected persons. These findings contrast with those of Hay et al. [7], who, using a PCR technique less sensitive than ours, identified T. pallidum DNA in CSF from 7 of 14 HIV-infected patients with symptoms and signs consistent with CNS disease versus none of 14 samples from HIV-infected persons without evidence of CNS disease. Two of the 7 PCR-positive patients had no history of syphilis and had negative serologic tests for syphilis, raising the possibility of false positives. Although the possibility of contamination of the PCR reaction was excluded, samples could have been falsely positive because of manipulation in a laboratory that contained T. pallidum, a potential problem that was avoided in our study.

It remains possible that a few of our CSF samples could have contained small numbers of T. pallidum. This may have been more of a problem for smaller samples or samples that were thawed prior to long-term storage at −70°C [4], although we did not observe a change in the sensitivity of our assay when positive control samples were frozen and thawed twice. The 95% CI for our estimate of 0 of 81 (0–3.7%) suggests that up to three of our samples might have been falsely negative.

In our study, CSF T. pallidum DNA PCR did not provide additional information beyond conventional CSF analysis. In different studies, T. pallidum DNA has been detected in otherwise normal CSF from patients with early and late syphilis [3, 5] and has not been detected in CSF from patients with neurosyphilis confirmed by reactive CSF-VDRL [6, 7]. However, the number of patients studied has been small. Thus far, the clinical implications of detection of T. pallidum DNA in CSF by PCR appear to be similar to those of detection of T. pallidum in CSF by the rabbit infectivity test (RIT) [10]. In the absence of contamination, a positive result indicates that the CNS has been invaded by the organism, and treatment is often recommended, particularly if the patient is also HIV-infected [1]. Conversely, a negative PCR or RIT result for a CSF sample does not completely exclude the possibility of neurosyphilis.

Despite its limitations, PCR-based detection of T. pallidum DNA in CSF is a relatively simple and rapid technique that could easily be applied in clinical settings. Further prospective study is needed to determine the role of detection of T. pallidum DNA in CSF in the diagnosis and treatment of neurosyphilis.

References

Role of Bird Migration in the Long-Distance Dispersal of *Ixodes dammini*, the Vector of Lyme Disease

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**Materials and Methods**

*Study site.* Appledore Island (33.6 hectares) is in the Isles of Shoals archipelago, 9.7 km off the Maine–New Hampshire coast...