Diagnosis of California (La Crosse) Encephalitis by Precipitin Techniques: a Prospective Study

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Counter electrophoresis (CEP) and immunodiffusion (ID) were evaluated prospectively as methods for the early and rapid laboratory diagnosis of California encephalitis (CE). CEP and ID studies were done on paired sera from 127 patients with acute central nervous system infections. After the precipitin tests were completed, conventional hemagglutination-inhibition, neutralizing, and complement fixing antibody titers were measured. The CEP system detected antibodies in 7 (41%) of 17 CE patients during their acute illness and in all 17 patients during convalescence. The ID method was less sensitive; 3 of 17 acute sera and 16 of 17 convalescent sera were ID positive. Comparative precipitin studies indicated that La Crosse virus was the infecting California group subtype in all 17 CE patients. Because CEP can be performed in 1.5 h, is at least as sensitive as hemagglutination-inhibition, neutralizing, and complement fixing tests, and can detect prospectively 41% of CE patients during their acute illness, it is recommended as a rapid diagnostic test for CE.

California encephalitis (CE) has been recognized in the United States since 1960, and more than 500 cases of CE have been reported from 18 states during the past decade (2, 4). Sixty-six of these cases have occurred in Minnesota children (2). Early and rapid diagnosis of CE is especially important, since the initial clinical course may closely resemble Herpesvirus hominis encephalitis (2, 5). In a previous retrospective study, we reported that immunodiffusion (ID) and counter electrophoresis (CEP) were rapid methods that would be useful for the early detection of some CE patients (1). The purpose of the present investigation was to evaluate these methods prospectively.

MATERIALS AND METHODS

Five low-passaged California group arboviruses, which included four prototypes and one Minnesota La Crosse strain, were used as seed viruses to prepare ID and CEP antigens. Prototype La Crosse, snowshoe hare, Jamestown Canyon, and Trivittatus viruses were kindly supplied by W. H. Thompson, Madison, Wisconsin. La Crosse strain Z-7-71 was isolated by us from a pool of four Aedes triseriatus mosquitoes collected in Zumbra Ridge, Minnesota, in August, 1971. It was identified as a La Crosse subtype by comparative tissue culture neutralization (NT) and complement fixation (CF) testing (3), using antisera obtained from W. H. Thompson and the National Center for Disease Control. The precipitin and neutralizing antigens and the control rabbit antisera for ID and CEP studies were made as previously detailed (1).

Throat swabs, stools, cerebrospinal fluids, and brain biopsy specimens were processed for virus isolation as previously described (2). If a virus was isolated, the patient's sera were tested for Nt antibodies to the homologous virus.

Venous blood was drawn from patients by sterile techniques and allowed to clot at room temperature. The sera were separated by centrifugation and stored in glass screw-capped vials at −70°C until tested. During the months of June through October, 1973, acute sera from 127 Minnesota patients with clinical diagnoses of central nervous system infections were tested by ID and CEP as soon as they were received. After the convalescent serum was obtained, the acute and convalescent sera were tested simultaneously for precipitin antibodies. When the precipitin studies were completed, the paired sera were tested for La Crosse hemagglutination-inhibition (HI), Nt, CF, Herpesvirus hominis type 1 CF, mumps CF, and Western equine encephalitis HI antibodies using standard procedures (3). The serologic diagnosis was based on a fourfold or greater change in HI, Nt, and/or CF titers. Comparative ID and CEP were done on the sera from CE patients to determine the infecting California arbovirus subtype as previously described (1).

The ID tests were done using a micro-slide technique described by Wellings, Sather, and Hammon with minor modifications (6). The buffer was tris(hydroxymethyl)aminomethane (Tris)-buffered saline,
pH 7.4. ID was carried out at room temperature on undiluted sera. Slides were read after 24 and 48 h against a dark background with indirect light and were graded 0 to 4+ according to precipitin arc intensity. Known negative and positive rabbit and/or human sera were included as controls for each test. Normal suckling mouse brain was used as a control antigen to ensure that none of the positive sera contained precipitin antibodies to mouse brain tissues.

The CEP plates were prepared by pouring 6 ml of 1% agarose in Tris-buffered saline, pH 8.0, into each plate. Wells for antigens and antisera were cut 5 mm apart with a 3-mm diameter metal punch. Twelve pairs of wells were cut for each plate. Sera were tested undiluted. Antigens were diluted 1:2 with Tris-buffered saline, pH 8.0. CEP was performed in an Abbott electrophoresis chamber for 1 h by applying 45 mA of current per plate. The buffer in the chamber was a 0.04 M barbital-acetate in 0.1 M sodium chloride, pH 8.2. The plates were read immediately after being tested and were graded in the same manner as the ID slides. The same controls as used in the ID system were included.

RESULTS

The 127 patients studied prospectively during the months of June through October 1973 are listed by laboratory diagnosis (Table 1). Seventeen (13%) of these 127 patients had California (La Crosse virus) encephalitis. The diagnosis was based both on the clinical findings of acute central nervous system infection and the conventional serologic results summarized in Table 2.

La Crosse virus was the most commonly recognized cause of acute viral central nervous system disease during the summer of 1973 in Minnesota (Table 1). Twenty-one patients had other viral agents associated with their acute central nervous system disease. Coxsackievirus A9 and mumps were the next most common viral agents, being responsible for five cases of meningoencephalitis each. The other viral agents listed were implicated less frequently, and no diagnosis could be made for 89 (70%) of the patients.

Seven patients had La Crosse precipitins in their acute sera by the CEP method, and 17 had La Crosse precipitins in their convalescent specimens. Using the ID system, only three patients had precipitins in their acute specimens and 16 were precipitin positive in their convalescent specimens. The serologic diagnosis of La Crosse virus encephalitis was confirmed in the 17 patients who had positive La Crosse CEP tests on the basis of a fourfold or greater change in HI, Nt, and/or CF titers. Comparative precipitin studies indicated that La Crosse virus was the California subtype responsible for CE in all 17 patients. All of the 110 patients who had negative La Crosse ID and CEP tests had negative HI, Nt, and/or CF tests.

Seven (41%) of 17 patients with La Crosse virus encephalitis had precipitins in their acute serum specimens by the CEP method. These seven positive specimens were collected from 0 to 17 days after the onset of illness (mean, 6.3

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TABLE 1. Patients with central nervous system infections studied prospectively for serum precipitins to La Crosse virus

| Laboratory diagnosis     | No. of patients | Positive La Crosse CEP | Positive La Crosse ID |
|--------------------------|-----------------|------------------------|-----------------------|
| La Crosse virus*         | 17              | 7                      | 3                     |
| Coxsackievirus A9        | 5               | 0                      | 0                     |
| Mumps                    | 5               | 0                      | 0                     |
| Herpesvirus hominis      | 3*              | 0                      | 0                     |
| Echovirus 9              | 2               | 0                      | 0                     |
| Miscellaneous viruses*   | 6               | 0                      | 0                     |
| No diagnosis             | 89              | 0                      | 0                     |

*See Table 2 for specific serologic results.
One brain biopsy isolate; two serologic diagnoses.
One each of coxsackievirus B3, echovirus 25, adenovirus, cytomegalovirus, subacute sclerosing panencephalitis, and a dual coxsackievirus A9-echo 11 infection.

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TABLE 2. Serologic diagnosis of California (La Crosse) encephalitis by HI, Nt, and CF titers

| No. of patients | HI (fourfold rise) | HI (fourfold decline) | Nt (fourfold rise) | CF (fourfold rise) |
|-----------------|--------------------|-----------------------|--------------------|--------------------|
| 12              | 12                 | 0                     | 12                 | 8*                 |
| 2               | 0                  | 2                     | 2                  | 2                  |
| 2               | 0                  | 0                     | 2                  | 2                  |
| 1               | 0                  | 0                     | 0                  | 1                  |

*Three not tested.
DISCUSSION

There are several features of CE that render clinical recognition difficult and, thus, make early and rapid laboratory diagnosis desirable. First, patients with CE may have an early clinical course which is indistinguishable from that of Herpesvirus hominis encephalitis (2, 5). Patients with Herpesvirus hominis encephalitis might be treated with toxic deoxyribonucleic acid antagonists that have not been shown to be effective against the California arbovirus group. A rapid diagnosis of CE would militate against the decision to use these antiviral agents. Also, the diagnosis of herpes encephalitis may require a brain biopsy and in one instance a Minnesota patient with California encephalitis was subjected to a brain biopsy because the working diagnosis was Herpesvirus hominis encephalitis (2). Early and rapid diagnosis of CE might spare these patients such invasive procedures.

Second, patients with CE frequently have a leukocytosis which may be as high as 30,000 cells per mm³, and they may also have a predominance of polymorphonuclear cells in their spinal fluid (2). The peripheral leukocytosis and presence of polymorphonuclear cells in the spinal fluid suggests bacterial meningitis, and the majority of our patients with CE initially have been treated with antibacterial agents. A rapid laboratory diagnosis of CE would enable the physician not to use or to discontinue antibacterial therapy.

In a retrospective study conducted on 40 patients with California encephalitis, we showed that precipitin antibody tests were useful for the laboratory diagnosis of CE and could detect between 36 to 44% of patients during the acute illness (1). Precipitin antibodies were particularly useful for early diagnosis because they were found only in patients with CE, whereas HI, Nt, and CF antibodies occurred in individuals without a history of CE (1). In addition, precipitin antibodies disappeared in all but one instance within a year after the onset of CE (1). Because CE has occurred only in the summer months in the United States (4), and because most patients with CE become precipitin negative within a year of illness, the presence of precipitin antibodies in an acute or convalescent specimen from a patient with a viral central nervous system infection is essentially diagnostic of CE.

Seven (41%) of 17 patients with CE in this present prospective study had precipitins in their acute sera. Thus, an early diagnosis of CE was made in those patients. The earliest that an acute serum was positive was the day of onset of illness. In that child, the physician was phoned and given the presumptive laboratory diagnosis. Because that particular patient had a clinical course which was believed to be most compatible with herpesvirus encephalitis, transfer of the patient to another institution with consideration of performing a brain biopsy had been planned. The patient remained at the community hospital following the presumptive diagnosis of CE and she made a rapid recovery from her meningoencephalitis after 3 days of intensive care.

The CEP test detected all of the 17 patients who had a subsequent laboratory diagnosis of CE based on conventional tests. Hence, the CEP system appears to be at least as sensitive as HI, Nt, and CF tests for the detection of CE antibody. However, in one patient in the present prospective study and in another in the retrospective study, it was found that precipitin antibodies developed late (8 to 16 weeks after onset of illness). If a viral diagnosis cannot be established using a convalescent serum obtained 3 to 4 weeks after the onset of illness, it might be of value to draw a third specimen 4 to 8 weeks later.

In this prospective study, the CEP method appeared to be more sensitive than the ID method. The ID system did not detect 4 of the 7 acute sera and 1 of the 17 convalescent sera which were CEP positive. Because the CEP system appears to be somewhat more sensitive than ID and because it can be performed in 1.5 h whereas ID requires 24 h, we consider the CEP method the laboratory test of choice for the rapid detection of CE antibody.

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