Viral Infections of the Central Nervous System in Spain: A Prospective Study

The aim of the study was to determine the incidence of viruses causing aseptic meningitis, meningoencephalitis, and encephalitis in Spain. This was a prospective study, in collaboration with 17 Spanish hospitals, including 581 cases (CSF from all and sera from 280): meningitis (340), meningoencephalitis (91), encephalitis (76), febrile syndrome (7), other neurological disorders (32), and 35 cases without clinical information. CSF were assayed by PCR for enterovirus (EV), herpesvirus (herpes simplex [HSV], varicella-zoster [VZV], cytomegalovirus [CMV], Epstein–Barr [EBV], and human herpes virus-6 [HHV-6]), mumps (MV), Toscana virus (TOSV), adenovirus (HAdV), lymphocytic choriomeningitis virus (LCMV), West Nile virus (WNV), and rabies. Serology was undertaken when methodology was available. Amongst meningitis cases, 57.1% were characterized; EV was the most frequent (76.8%), followed by VZV (10.3%) and HSV (3.1%; HSV-1: 1.6%; HSV-2: 1.0%; HSV non-typed: 0.5%). Cases due to CMV, EBV, HHV-6, MV, TOSV, HAdV, and LCMV were also detected. For meningoencephalitis, 40.7% of cases were diagnosed, HSV-1 (43.2%) and VZV (27.0%) being the most frequent agents, while cases associated with HSV-2, EV, CMV, MV, and LCMV were also detected. For encephalitis, 27.6% of cases were caused by HSV-1 (71.4%), VZV (19.1%), or EV (9.5%). Other positive neurological syndromes included cerebellitis (EV and HAdV), seizures (HSV), demyelinating disease (HSV-1 and HHV-6), myelopathy (VZV), and polyradiculoneuritis (HSV). No rabies or WNV cases were identified.

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Correspondence to: F. de Ory, Servicio de Microbiología Diagnóstica, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda 28220, Madrid, Spain.
E-mail: fory@isciii.es
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INTRODUCTION

The aseptic forms of meningitis, encephalitis, and meningoencephalitis are non-purulent inflammatory processes of the central nervous system (CNS), where initial assessment using the usual staining and culture of cerebrospinal fluid (CSF) is negative. Epidemiological studies indicate that viruses are the main identified cause of acute meningitis and encephalitis [Koskiniemi et al., 2001; Glaser et al., 2003; Kupila et al., 2006]. Of major importance are the enteroviruses (EVs), herpes simplex virus (HSV)-2 and varicella-zoster virus (VZV) in meningitis, and HSV-1 in encephalitis. Human immunodeficiency virus (HIV) has been identified as an important agent causing meningoencephalitis [del Saz et al., 2008].

In Spain, there have been some studies based on the detection of etiological agents, and on studies of antibodies which examine the involvement of viral agents in the production of meningitis and encephalitis. In the case of meningitis, the most frequent agents are EVs, particularly in children, followed by VZV and HSV, more frequently in adults [Tellez et al., 1989; Echevarria et al., 1997a; De la Loma et al., 2002]. Other pathogens detected in prior studies were mumps (MV) [Tellez et al., 1989], Toscana virus (TOSV) [Navarro et al., 2004; De Ory et al., 2009], West Nile virus (WNV) [Kaptoul et al., 2007], and lymphocytic choriomeningitis virus (LCMV) [De Ory et al., 2009]. As regards encephalitis, the information available in Spain is more limited. A previous study found that HSV and VZV were the most frequent agents while Epstein–Barr virus (EBV), cytomegalovirus (CMV), human herpes virus-6 (HHV-6), and EVs were the cause of a minor number of cases [Echevarria et al., 1997b].

Other neurological disease-producing agents are human adenovirus (HAdV) [Lema et al., 2005]. With regard to rabies, the pathognomonic hallmarks are not always apparent and it may present with general symptoms of encephalitis. Although the rabies virus (RV) was eradicated from the Spanish mainland and the islands in 1978, imported cases of canine rabies are still being reported on a regular basis in the Spanish North-African cities of Ceuta and Melilla. In 2004, one imported human case was declared in Ceuta [Strauss et al., 2005]. In addition, the European bat lyssavirus type 1 is endemic in some Spanish bat species and can cause rabies in man, not always accompanied by the characteristic symptoms [Vazquez-Morón et al., 2008].

Recently, the situation of encephalitis and meningoencephalitis surveillance in Europe, which highlighted the advisability of running studies on the etiological characterization of neurological syndromes in European countries, was analyzed. This would reveal the incidence of pathogens of concern for public health, including emerging viral infections [Donoso Mantke et al., 2008]. The aim of this study was to identify the viruses responsible for CNS infections, by testing for EVs, HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, MV, TOSV, HAdV, LCMV, Flavivirus (WNV) and tick borne encephalitis virus (TBEV), and Rhabdovirus.

MATERIALS AND METHODS

Design

Prospective observational study of acute viral CNS infections involving diagnostic virology laboratories at 17 hospitals from the Spanish Health National System. These hospitals serve a population of 7,572,771 inhabitants (6,762 million adults over 14 years of age and 810,771 children under 14), accounting for 17.17% of the Spanish population (17.9% of those over 14 and 12.9% under 14), according to data from the 2005 municipal census (http://www.ine.es).

Patients

A total of 581 cases classified at admission as meningitis, meningoencephalitis, or encephalitis was studied. At discharge the cases were definitively classified as acute cases of meningitis (340 cases), meningoencephalitis (91), encephalitis (76), febrile infant syndromes (7), and other conditions (32) (headache [2], cerebellitis [2], seizures [6], confusion [1], diploria [1], demyelinating disease [3], hypotension [1], leukoencephalopathy [1], cerebral lymphoma [1], meningeval lymphomatosis [1], myelitis [3], myelopathy [4] multi-neuritis [1], optic neuritis [2], paralysis [1], and polyradiculoneuritis [2]), diagnosed by internal medicine or neurology or pediatrics services in the hospitals participating from March 1, 2008 to February 28, 2009. In 35 of the cases, no clinical information was available. Informed consent was obtained from all patients (or guardians in the case of children under 14 years). For inclusion, all patients had to be immunocompetent, their CSF sample had to present lymphocytic pleocytosis >5 cells/mm³ and the sample had to be collected during the first week of onset of symptoms. Meningitis cases coursed with fever, headache,
and neck rigidity. For diagnosis of encephalitis, cases needed to present one or more clinical signs of focal cerebral involvement, an altered level of consciousness, neuropsychiatric clinical symptoms, or compatible radiological signs. Meningoencephalitis included signs or symptoms from both encephalitis and meningitis.

Clinical and Epidemiological Data

A form specifically designed for this purpose was completed in each case, recording demographic data (age, sex, seasonality of episode, length of hospitalization) and clinical data (presence of fever ≥38°C and its duration in days, neurological symptoms and association of systemic symptoms such as diarrhea, arthralgia, myalgia, lymphadenopathy, respiratory symptoms).

Samples

In all cases, CSF sample from the first week of onset of symptoms was available (minimum of 400 μl). Three hundred twenty-one serum samples from 280 cases were studied: two cases with three samples, 37 cases of acute and convalescent serum, acute serum only from 236 cases, and only convalescent serum in five cases.

Virological Study

This was run on the CSF samples, preparing two aliquots of 200 μl each. With the first one, the presence of at least HSV-1 and -2, VZV and EV by polymerase chain reaction (PCR) was determined in each hospital, using each laboratory’s own procedures. The second aliquot was mixed with a lysis buffer based on guanidinium thiocyanate (300 μl; MagnaPure LC Total Nucleic Acid Isolation Kit, Roche Diagnostics, Mannheim, Germany), kept at −20°C for subsequent submission to the National Microbiology Centre (CNM), where the nucleic acids were automatically extracted (MagnaPure LC, Roche Diagnostics) and processed in a multiplex PCR assay for EV, HSV, VZV, CMV, EBV, and HHV-6 [Casas et al., 1997]. Where the result was negative, samples were processed by PCR for HAdV [Avellón et al., 2001], MV [Palacios Poggio et al., 2000], RV [Echevarría et al., 2001], TOSV [Sánchez-Seco et al., 2003; Pérez-Ruiz et al., 2007], for the identification of as yet undescribed members of the Herpesviridae family (using a generic PCR); Flavivirus [Sánchez-Seco et al., 2005]; Arenavirus (or LCMV) [Ledesma et al., 2009]; Lyssavirus [Vázquez-Morón et al., 2006]; RV, and Dimarhabdovirus. In samples which tested positive for HSV, a typing test based on the polymerase gene was used.

Cases which showed positive results at the sender laboratory and/or in the CNM were considered positive.

Samples positive for EV, HAdV, or MV were genotyped by assays described previously [Casas et al., 2001, 2005; Palacios et al., 2005; Cabrerizo et al., 2008]. Bacterial and fungal aetiology was discarded in all cases.

Serological Study

The following tests were performed depending on sample availability: antibodies to HSV and VZV by complement fixation (CF), IgM antibodies to HSV and VZV, and IgG and IgM antibodies to MV and TBEV by ELISA (Enzygnost, Siemens, Germany), IgG antibodies to HSV-1 and HSV-2 by immunoblot (Virotech, Rüsselheim, Germany), IgG and IgM antibodies to TOSV, by ELISA (Diesse, Monteriggioni, Siena, Italy); IgG and IgM antibodies to WNV by ELISA (Focus, Cypress, CA) and IgG and IgM antibodies to LCMV by indirect immunofluorescence as described [De Ory et al., 2009].

In one case serum/CSF paired samples with 26 days of infection were available. The intrathecal synthesis of IgG antibodies to HSV and VZV was examined, establishing the ratio of antibody to albumin [Echevarría et al., 1990].

RESULTS

Direct Viral Detection by PCR

Result were obtained for 566 of the 581 samples studied, with positive results in 250 cases using PCR (44.2%): this was 161 for EVs (66.4% of positives; 28.4% of the total); 31 for HSV-1 (12.4% and 5.5%); 28 for VZV (11.2% and 4.9%); three for HSV-2 (1.2% and 0.5%); one HSV-non typed (nt; 0.4% and 0.2%); four CMV (1.6% and 0.7%); three EBV (1.2% and 0.5%); two HHV-6 (0.8% and 0.4%); two TOSV (0.8% and 0.4%); two HAdV (0.8% and 0.4%); one MV (0.4% and 0.2%); one LCMV (0.4% and 0.2%); and mixed infections in 11 cases (4.4% and 1.9%): five EVs, one HSV-1, one VZV, and four EVs plus HSV-nt. Thus, including mixed infections, 170 EV, 32 HSV-1, 5 HSV-nt, and 29 VZV were identified. No cases due to RV, WNV, or herpes virus other than those already known were identified.

Genotype Characterization

The samples which tested positive for EV, HAdV, and MV were genotyped. As regards EV, 132 were positive in the CNM, of which 70 were genotyped (53%). Furthermore, the only sample which identified MV positively by PCR was characterized as genotype G1. The two cases of HAdV belonged genetically to serotype HAdV-5.

SeroLOGY

For HSV and VZV 315 samples were studied from 277 cases: one case with three samples; 36 cases of acute and convalescent sera; 235 cases of acute serum
only, and five cases of convalescent only. Fourteen cases of HSV-nt infection (5.1% of those studied) were serologically diagnosed; four cases by seroconversion, two cases by presence of IgM, and eight cases by high titres in CF. Two cases were positive for HSV infection but negative in PCR. With regard to specific response to HSV-1, the result was positive in 11 cases, five of them simultaneously with detection of the virus by PCR. Finally, one case of infection with HSV-2, simultaneously with identification of the virus by PCR was diagnosed.

Seventeen cases (6.1%) turned out to be VZV-positive: nine with a positive PCR, seven were positive only by serology, and one last case was diagnosed by specific intrathecal IgG production and by serology. In addition, three cases with positive direct detection of other viruses (two EV and one HSV-1) showed positive VZV serology.

MV IgM was studied in 321 samples from 280 cases, with positive results in four cases (1.4%): one with G1 genotype identification by PCR, and three with negative PCR. Three other cases with positive direct detection of EV (two cases) and VZV showed positive results. In addition, IgG-MV was studied in 221 cases, obtaining positive results in 187 cases (84.6%). High titres (≥3,500) were identified in 49 cases (22.2% of the total, i.e., 26.2% of the positives).

TOSV IgG and IgM were analyzed to in 318 serum samples from 278 patients, diagnosing one case with reactivity to both isotypes (0.4%) and positive PCR. The presence of specific IgG was detected in 18 cases out of the 277 remaining cases (6.5%).

LCMV IgG and IgM antibodies were studied to in 270 samples from 235 cases, obtaining positive IgM in three cases (1.3%), one with a positive PCR in CSF for LCMV, a second with negative PCR, and a third from a mixed infection with direct identification of EV. When analyzing IgG, 10 positive cases were obtained from those not revealing recent infection (4.3%). IgG and IgM antibodies to WNV were determined in 303 serum samples from 267 patients. No cases of recent infection with the virus were diagnosed, however five positive IgG samples were identified (1.9%). Finally, with regard to TBEV, 138 samples were studied from 121 cases. No cases indicative of recent infection were identified but IgG was detected in three cases (2.5%).

**Overall Results**

Overall results, including both the results for PCR, such as serology in serum and CSF, are presented in Table I. A total of 270 cases were finally characterized (46.5% of the 581 cases received).

**Etiological Characterization of Cases of Meningitis, Meningoencephalitis and Encephalitis**

Both final results obtained by clinical picture and those obtained by both clinical characteristics and age group are shown in Tables II and III.

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**TABLE I. Total Positive Results by Diagnostic Approximation (PCR and/or Serology)**

|                  | PCR in CSF alone | PCR in CSF and serology | Abs in CSF and serology | Serology alone | Total |
|------------------|------------------|-------------------------|-------------------------|---------------|-------|
| EV               | 161              |                         |                         |               | 161   |
| HSV              | 26               | 5                       | 6                       | 37            |
| HSV-nt           | 1                | 2                       | 3                       |               |
| HSV-2            | 2                | 1                       | 3                       |               |
| VZV              | 19               | 9                       | 1                       | 7             | 36    |
| MV               | 1                | 3                       | 4                       |               |
| CMV              | 4                | 4                       |                         |               |
| EBV              | 3                | 3                       |                         |               |
| HHV-6            | 2                | 2                       |                         |               |
| LCMV             | 4                | 1                       | 1                       | 2             |
| TOSV             | 1                | 1                       |                         |               |
| HAdV-5           | 2                | 2                       |                         |               |
| Mixed infections | 4                | 7                        |                         |               |
| **Total**        | 226              | 24                      | 1                       | 19            | 270   |

*aNo serology was undertaken for EV, HHV-6 and HAdV-5.
*bCases with mixed infections are listed in Table IV.
*cIncluding five EV, one VZV, and one HSV-1 detected by PCR.

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**TABLE II. Results Obtained, by Syndrome**

|       | M | ME | E | Others* | Total |
|-------|---|----|---|---------|-------|
| EV    | 149| 3  | 2 | 7 (6)  | 161   |
| HSV   | 1  | 2  |   |         | 3     |
| HSV-1 | 3  | 16 | 15| 3       | 37    |
| HSV-2 | 2  | 1  |   |         | 3     |
| VZV   | 20 | 10 | 4 | 2       | 36    |
| CMV   | 2  | 2  |   |         | 4     |
| EBV   | 3  | 3  |   |         | 3     |
| HHV-6 | 1  | 1  |   |         | 2     |
| MV    | 3  | 1  |   |         | 4     |
| TOSV  | 2  | 2  |   |         | 2     |
| HAdV  | 1  | 1  |   |         | 2     |
| LCMV  | 1  | 1  |   |         | 2     |
| Mixed | 6  | 3  | 2 | 11      |       |
| Positive | 194 | 37 | 21| 18 (6)  | 270   |
| Negative| 146| 54 | 55| 21 (1)  | 276   |
| **Total** | 340| 91 | 76| 39 (7)  | 546   |

In 35 cases, clinical data were not available.
M, meningitis; ME, meningoencephalitis; E, encephalitis.
*In brackets are included the cases of febrile infant syndrome.
*bCases with mixed infections are listed in Table IV.

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and one HSV-nt: 0.5%); EBV (three cases, 0.9%, 1.6%), CMV (two cases, 0.6% 1.0%); HHV-6 (one case, 0.3% and 0.5%); MV (three cases, 0.9%, 1.6%); TOSV (two cases, 0.6%, 1.0%) and HAdV and LCMV (one case, 0.5%, 0.3% each). Double positives were found in six cases (1.8% and 3.1%), as listed in Table IV.

Among the cases of meningoencephalitis, HSV-1 was the most frequent cause, both overall (16 cases, 17.6% of the total, 43.2% of positives; Table II) and in all age groups, followed by VZV (10 cases, 11.0% and 27.0%; Table III). Importantly, in 16–60 and >60 age groups both HSV and VZV caused respectively 35% (14/40) and 36% (9/25) of cases studied. EV caused three cases (3.3% and 8.1%); CMV, two cases (2.2% and 5.4%); and HSV-2, MV, and LCMV one case each (1.1% and 2%). Double positives were found in three cases (3.3% and 8.1%), as listed in Table IV.

Among the cases of encephalitis, the most common cause was HSV-1 (15 cases, 19.7% of the total, 71.4% of positives), followed by VZV (four cases, 5.3% and 19.1%) and EV (2.6% and 9.5%). All positive cases in patients older than 16 years were due to HSV-1 and VZV (Table III). No multiple infections were detected.

In both meningoencephalitis and encephalitis etiological diagnosis was not achieved in younger age groups.

Six cases of febrile syndrome in infants revealed infection by EV.

The results obtained in cases with other clinical pictures are shown in Table V. Positive results were obtained in 12/32 (37.5%). Of the cases with etiological identification, one case of conjunctivitis associated with cerebellitis was characterized, caused by HAdV-5; three cases with seizure associated with HSV (two cases with HSV-1 and one with HSV-nt) out of the six studied; two cases of demyelinating disease (one associated with HSV-1 and another with HHV-6); out of three analyzed, one case of VZV myelopathy, out of four studied, and one case of polyradiculoneuritis due to HSV-nt, from two analyzed.

**DISCUSSION**

A total of 270 positive cases were identified (46.5% of those studied). Among the cases of meningitis, the most frequent cause was EV, while among the cases of meningoencephalitis and encephalitis the most frequent cause were the HSV and VZV. These results basically concur with studies run in Spain on both meningitis [Tellez et al., 1989; Echevarria et al., 1997a; De la Loma et al., 2002] and encephalitis [Fernandez-Viladrich et al., 2007].

In children the most frequent cause for meningitis was EV, as has been described in Spain [Tellez et al., 1989]. The relative participation of both HSV and VZV in causing meningitis increases with age. The findings in older people are similar to those recently described [Delerme et al., 2009]. EV was the most frequent cause, being HSV and VZV also important ones.

As regards encephalitis, the information available is more limited. A study conducted from 1989 to 1995 in Spain found that HSV and VZV were responsible

| Age group | EV | HSV | HSV-1 | HSV-2 | VZV | HHV-6 | EBV | CMV | O | T |
|-----------|----|-----|-------|-------|-----|-------|-----|-----|---|---|
| <15       | 23 | 12  | 1     | 1     | 0   | 0     | 0   | 0   | 0 | 0 |
| 1–15      | 83 | 1   | 1     | 3     | 4   | 127   | 1   | 1   | 2 | 12 |
| 16–60     | 34 | 2   | 2     | 12    | 1   | 1     | 6   | 131 | 2 | 10 |
| >60       | 1  | 1   | 1     | 1     | 2   | 22    | 0   | 4   | 1 | 40 |
| Total     | 141| 3   | 2     | 16    | 1   | 3     | 2   | 131 | 3 | 15 |

O, other viruses (MV, TOSV, HAdV, LCMV); T, total.

**TABLE IV. Cases With Mixed Infections**

| Age group | Sex | Diagnosis         | CSF PCR | Serology        |
|-----------|-----|-------------------|---------|-----------------|
| <15       | F   | Meningitis        | EV      | LCMV (IgM)      |
| <15       | F   | Meningitis        | EV = HSV-nt | No sample |
| <15       | M   | Meningitis        | EV      | VZV (IgM)       |
| <15       | M   | Meningitis        | EV      | MV (IgM)        |
| <15       | M   | Meningitis        | EV = HSV-nt | No sample |
| <15       | M   | Meningitis        | EV = HSV-nt | No sample |
| ND        | ND  | Meningoencephalitis| EV      | VZV (IgM)       |
| 15–60     | F   | Meningoencephalitis| EV      | MV (IgM)        |
| 15–60     | F   | Meningoencephalitis| HSV-1   | VZV (SC-IgM)    |
| ND        | ND  | Cerebellitis      | EV      | MV (IgM)        |
| 15–60     | F   | Diplopia          | EV = HSV-nt | No sample |

ND, no data; SC, seroconversion.

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Adult cases of meningitis may be caused by many agents, of which HSV and VZV are the main ones, with HSV-1 and VZV amongst meningoencephalitis. Several reports have provided us the identification of other agents as cause of CNS infection in Spain. Although HIV has been identified as an important agent causing meningoencephalitis, its importance decreasing with increasing vaccine coverage [Echevarria et al., 1997a]. In the present study, four cases were identified, figure coinciding with low circulation of the virus at the time when the cases were collected (in 2008 there were reported 2,044 cases in Spain, and 3,741 in 2009) [Boletin Epidemiologico Semanal, 2009]. Furthermore, only one case of CMV by direct detection has been identified, characterized as G1, the most prevalent genotype in previous seasons with the largest circulation of MV [Echevarria et al., 2010]. Furthermore, quantification of specific IgG in serum has been suggested as a marker of recent infection in periods of viral circulation [Sanz et al., 2006]. On this point, high titres (≥1/3,500) were seen in about 25% of cases. However, considering the conventional indicators of recent infection (PCR and IgM), the high titres obtained in this study may reflect infections in previous seasons, rather than current infection.

LCMV has been identified in rodents in Spain [Ledesma et al., 2009], being responsible for 1.5% of cases negative for frequent aseptic meningitis agents [De Ory et al., 2009]. In this study, two cases of meningitis (one with simultaneous detection of EV), and another of meningoencephalitis, have been identified. The three cases were located in the south-east of the Spanish mainland, one in Granada and the other two in Murcia. The first, which was diagnosed by both PCR and serology, was in a deprived urban area, where another case caused by the virus was diagnosed 1 year later [Pérez-Ruiz et al., 2012], thus identifying an area of risk for this virus. On the other hand, the global LCMV IgG seropositivity obtained in the cases studied is equivalent to what has been previously documented in a similar sample [De Ory et al., 2009]. The current study shows the circulation of the virus in Spain, with the implications that may arise from the seriousness of infection in patients with transplants from apparently healthy donors [Fischer et al., 2006].

With respect to TOSV, two cases were diagnosed, both in south-east Spain, the number of cases being lower than obtained in a previous report [Navarro et al., 2004]. A study on sera from cases which were negative for the common agents which cause meningitis, confirmed that TOSV causes 6% of cases, with major year-on-year variations (from 1.2% to 12.5%) [De Ory et al., 2009]. Possible causes of the low incidence obtained may be the climatic differences in terms of temperature and humidity in different years, which may affect the circulation of the vector (Phlebotomus perniciosus).

Flaviviruses are known to cause neurological syndromes. TBEV and WNV have been identified in Europe, in central Europe (TBEV) and in the Mediterranean basin (WNV). To date there have been no known cases of infection by TBEV in Spain, and only sporadic cases of neurological disease caused by WNV [Kaptoul et al., 2007; Jesús-De La Calle et al., 2012] have been documented. In this study, no cases due to these viruses were identified, although some individuals were found to be IgG positive. Specifically, WNV seroprevalence obtained in Catalonia (4.4%, results not shown) was higher than documented in a previous study [Bofill et al., 2006]. WNV and TBEV belong to the genus Flavivirus, and show a high degree of cross-reaction between each other and other members of the genus (dengue, yellow fever, and others). Thus,

TABLE V. Etiological Characterization of Cases With Other Clinical Conditions

| Age | Sex | Diagnosis          | Result       |
|-----|-----|--------------------|--------------|
| <15 | Female | Headache               | EV-nt        |
| 15–60 | ND | Headache             | VZV          |
| ND | ND | Cerebellitis         | EV + MV      |
| ND | Female | Cerebellitis + conjunctivitis | HAdV-5 |
| 15–60 | Male | Seizure             | HSV-1        |
| 15–60 | Male | Seizure             | HSV-nt       |
| 15–60 | Female | Diplopia             | HSV-nt + EV  |
| 15–60 | Female | Demyelinating disease | HSV-1 |
| ND | ND | Demyelinating disease | HSV-6       |
| 15–60 | Female | Myelopathy           | VZV          |
| ND | Male | Polyradiculoneuritis | HSV          |

ND, no data.
the reactivity detected in some of these cases may be due to infection by another or other flaviviruses circulating in Spain.

Of the 51 known HAdV serotypes, types -2, -3, -7, -26 and -31 and -49 [Schnurr et al., 1995; Ohtsuki et al., 2000; Dubberke et al., 2006; Nagasawa et al., 2006; Vincentelli et al., 2010] have been linked to neurological infection. HAdV has been identified as a complication of pneumonia as a result of disseminated infection in immunocompromised patients; the infection rarely presents primarily as a CNS disease [Studahl et al., 1998]. HAdV-5, identified in this study as the cause of one case of cerebellitis with a history of conjunctivitis, and another of meningitis, is usually recognized as a major cause of respiratory infection in infants (bronchiolitis) [Belys et al., 2009].

Apart from this HAdV-5 cerebellitis case, other medical conditions of interest have been diagnosed (Table V). Three cases of seizures associated with HSV (two cases with HSV-1, and one with HSV-nt), out of six studied were identified. This relationship has previously been described, confirming HSV in 40% of epilepsy cases [Sanders et al., 1997]. Otherwise, the findings of HSV-1 and HHV-6 in cases of demyelinating disease can be considered nonspecific, as reported previously [Gordon et al., 1996]. The relationship of VZV with cases of myelopathy has been described previously [Morita et al., 2003]. Finally, infection with HSV in cases of polyradiculoneuritis is considered a rare finding [Cosson et al., 2002].

Serological tests for EV for the diagnosis of neurological infection are influenced by cross-reactions [Miao et al., 2009], and the large number of EV which can give rise to these diseases. As genomic amplification assays perform adequately, diagnosis is based almost exclusively on PCR. By contrast, in infections by other viruses, whose serological diagnosis has been more decisive than in the case of EV, the two diagnostic approaches have been seen to be complementary, improving the total number of cases diagnosed, as in the case of HSV, VZV, TOSV, LCMV, and MV in this study. However intrathecal production of antibodies was not properly explored, since no paired serum/CSF samples taken more than 10 days after onset were available. This approach showed to be an excellent complementary tool to direct diagnosis for encephalitis [Echevarría et al., 1990; Ambrose et al., 2011].

Dual infection in the central nervous system is a not frequent issue, and seems to be mainly related to herpesviruses. HSV-1 and VZV were simultaneously diagnosed in encephalitis patients by PCR and serology (in CSF and serum) [Casas et al., 1996], and CMV and HSV and CMV and EBV dual infections were described in two aids patients showing respectively encephalitis and polyradiculomelitis [Casas et al., 1999]. It has been difficult to find an explanation to mixed infections shown in Table IV. In one case acute and convalescent serum sample was available, allowing the confirmation of both HSV-1 and VZV infections. Serum sample from the four patients with double PCR result of EV and HSV-nt was not available to confirm at least the HSV infection; additionally, in the cases showing IgM reactivity (LCMV [one case], MV [three cases], or VZV [two cases]) no further analysis were done, since no follow-up serum sample was available.

The current study has the strong point of being a prospective study, conducted in Spanish Health National System public hospitals, serving a major proportion of the Spanish population; thus the results obtained are a reflection of the true incidence of these microorganisms in the production of neurological syndromes in Spain. A potential limitation, however, is that the cases included come from requests by clinics made to the laboratory; consequently the real circulation of these agents in Spain may actually be underestimated.

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