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Relation between Epstein-Barr virus and multiple sclerosis: analytic study of scientific production

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Abstract Numerous studies have been carried out to determine whether infection by the Epstein-Barr virus (EBV) can be considered as a risk factor for multiple sclerosis (MS). This work is a meta-analysis of case-control observational studies published before January 2009 aimed at assessing the degree of association between EBV and MS infections. A Medline electronic database search was carried out using “Epstein-Barr virus” and “multiple sclerosis” as keywords, from which we selected 30 published studies that met our methodology criteria. We found an association between MS and an exposure to EBV, studied by determining the anti-VCA IgG antibodies (odds ratio [OR]=5.5; 95% confidence interval [CI]=3.37–8.81; $p<0.0001$), anti-complex EBNA IgG (OR=5.4; 95% CI=2.94–9.76; $p<0.0001$) and anti-EBNA-1 IgG (OR=12.1; 95% CI=3.13–46.89; $p<0.0001$). No significant association could be found when studying anti-EA IgG (OR=1.3; 95% CI=0.68–2.35; $p=0.457$), EBV DNA in serum (OR=1.8; 95% CI=0.99–3.36; $p=0.051$) and DNA in brain tissues and in cerebrospinal fluid (CSF) (OR=0.9; 95%

CI=0.38–2.01; $p=0.768$). This meta-analysis detected an association between infection by EBV and MS through the investigation of antibodies, mainly anti-EBNA-1, anti-complex EBNA and anti-VCA IgG.

Introduction

Multiple sclerosis (MS) is a central nervous system (CNS) disease characterised by inflammation, demyelination, axonal degeneration and gliosis. Epidemiology studies show that it appears in genetically prone patients, in whom an unknown environmental factor would affect and trigger an alteration of the immune processes [1]. Infections caused by the Epstein-Barr virus (EBV) may be amongst such environmental factors. This virus is widely spread amongst the population, causing latent infections with continuous exacerbations and, finally, it shows immune-modulating activity [2]. The epidemiology of infectious mononucleosis (IM) and MS [3] are also very parallel, and it has been observed that individuals who have suffered late IM present a higher risk of developing MS [4–7] and the event is preceded by an increase in the specific antibodies many years before [8–10]. A recent study found evidence of EBV infection in a substantial proportion of brain-infiltrating B cells and plasma cells in nearly 100% of the MS cases examined. Ectopic B cell follicles forming in the cerebral meninges of some cases were identified as major sites of EBV persistence [11]. However, the debate over whether the association between EBV infection and MS is a coincidence or if, on the contrary, there is a causal relationship, single or multiple, is open. In spite of all the research carried out, most of the studies do not allow definite conclusions if they are individually considered.

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Ascherio and Munch [8] performed a systematic review of case-control studies comparing EBV serology in MS patients and controls. This study has consistently shown that the EBV seropositivity rate in MS patients is higher than in controls. Other meta-analysis concludes that EBV infection manifesting as IM in adolescents and young adults is a risk factor for MS [12]. Ascherio and Munger [13] found that MS risk is about 10-fold higher among individuals who experienced an undiagnosed EBV infection during childhood, and at least 20-fold greater among individuals who developed IM. The work of Holmøy [14] concluded that vitamin D deficiency and EBV infection are both very common population-wide events, and could be involved in MS pathogenesis. Finally, other authors [5, 15] in two well-designed cohort studies found increased relative risks of MS in subjects with IM, but the results from other studies were unconvincing [16].

Based on all of the above, this study located all international publications dated before January 2009 that analysed the relationship between EBV infection and MS, and they described a material and a well-defined method for each serologic or molecular determination. A meta-analysis and a quality study was carried out on the results obtained, so as to arrive at overall conclusions available to the date indicated, on the relationship between the virus and the disease.

Method

The Medline database was used to select the articles and an open search was carried out using “Epstein-Barr virus” or “EBV” or “human herpesvirus 4” or “HHV-4” or “infectious mononucleosis” or “environmental risk factors” and “multiple sclerosis” or “MS” as keywords. Thus, 251 studies were reliable, which were published before January 2009. Reviews were not considered. We selected only papers published in Spanish or English to analyse the relationship between the EBV and MS using a material and a well-described methodology. Not considered were those papers that did not show correct and explicit account of the results. Finally, we selected 30 studies [4, 10, 17–44] out of the 251 publications (see Table 1). As an additional measure to avoid possible losses, we also examined the bibliography of each paper, but we did not find any more studies in addition to the 30 papers initially selected.

The meta-analysis has a qualitative and a quantitative component. The former is an epidemiologic description of the papers, considering the individual studies as the research subject. For each serologic or molecular determination in each publication, both individually and in total, the following data were obtained: the odds ratio

(OR), confidence interval (CI) of the OR at 95%, statistical significance of the analysis and weight of the publication.

We used the DerSimonian and Laird [45] method to calculate the total, because it allows total estimates that are less affected by heterogeneity in the studies. The heterogeneity of the studies was determined using Cochran’s Q statistic method. Additionally, a measure of the variability of the total OR was employed, due to the heterogeneity of the studies: Higgins I^2 . We considered that no relationship existed between EBV exposure and the presence of MS when the CI at 95% included the unit [46].

Finally, we analysed the quality of the publications. We used the Newcastle-Ottawa [47] case and control point scale (NOS). To get to know whether there was any relationship between the quality of the article in the above-mentioned scale and its OR calculation, a meta-regression was carried out using the restricted maximum likelihood (REML) method in those cases in which the number of publications analysed was equal to or more than 3.

Data obtained from the various studies were analysed using the STATA Release 10.1 statistical package.

Results

Anti-VCA IgG determination

Among a total of 13 studies evaluating the anti-VCA IgG (Table 2), there was a single study in which such determination was made on cerebrospinal fluid (CSF) [23] and all of the remaining investigations were in serum [10, 17–19, 22, 24, 34–38, 43]. The percentage of cases positive for this determination (99–100%) was greater than among controls (81–98%), except in the study of Bray et al. [23]. In work performed among paediatric subjects, there was also a higher percentage of positive cases, but with differences more accentuated than in adults [17–19]. The study performed by Zivadinov et al. [43] was the only one in which the proportion of positive results was higher in controls than in cases. The study with the highest OR was published by Pohl et al. [19] and that with highest weight was published by Bray et al. [23], both of which indicate the existence of an association between the virus and the disease.

Comparing the ORs of the studies, we obtained $\chi_{\text{exp}}^2 = 19.74$, 12 g.l., $p = 0.072$, which indicates a trend towards difference, although not significantly. The global OR was 5.5 (95% CI = 3.37–8.81; $p < 0.0001$), indicating significant association between MS and exposure to the virus.

Individually considered, the studies with the highest quality were those by Myhr et al. [34], which stated a

Table 1 List of studies included in the meta-analysis

| Study | Cases | | | Controls | | | Sample/Test |
|-----------------------------------|---|--|---------------------|----------------------------|--|-----------------------|-------------------------------|
| | Diagnostic | Age Mean (SD) and/or range | F/M ratio | Diagnostic | Age Mean (SD) and/or range | F/M ratio | |
| Alotaibi et al. 2004 [17] | 30 MS | 13.4 (±3.63) | 1.31:1 | 90 healthy subjects | 13.37 (±3.62) | 1.57:1 | SR/ELISA |
| Alvarez et al. 2000 [20] | 102 RRMS-NAE | Female: 34.75 (21–57) Male: 36.34 (22–63) | 70/32 | 53 bone marrow donors | 10.30 (±3.78) | 0.66:1 | MC/PCR and n-PCR |
| Alvarez-Lafuente et al. 2008 [21] | 48 MS | 32.6 | 34/14 | 102 haemotherapy | Female: 34.75 (21–57) Male: 36.34 (22–63) | 70/32 | MC/PCR and n-PCR |
| Ascherio et al. 2001 [22] | 144 MS | – | 144/0 | 23 ONIND | 43.7 | 15/8 | CSF/RT-PCR |
| Banwell et al. 2007 [18] | 131 RRMS 5 SPMS 1 Marburg variant | 14.1 (2.2–19.6) | 1.54:1 | 21 ONIND | 39.4 | 14/7 | SR/IF |
| Bray et al. 1992 [23] | 266 MS | – | – | 288 healthy subjects | – | 288/0 | SR/ELISA |
| Bray et al. 1983 [24] | 313 MS | 16–>45 | – | 47 healthy subjects | – | – | SR/ELISA, IF and Western blot |
| Buljevac et al. 2005 [25] | 54 RRMS | 39.3 | – | 49 ONDND | – | – | SR/IF |
| Denne et al. 2007 [26] | 6 MS | 13.3 (±2.6) (10–17) | 5/1 | 52 healthy subjects | – | – | PS/ELISA |
| Ferrante et al. 2000 [27] | 26 RRMS-AE | 31 (20–46) | 17/9 | 57 OND | 9.2 (±5.1) | 30/27 | CSF/PCR |
| Haahr et al. 2004 [4] | 53 MS 100 newly diagnosed MS | 28.3 (21–32) 27.7 (20–34) | 49/4 73/27 | 18 healthy subjects | 29 (19–36) | 12/6 | MC/n-PCR |
| Hay and Tenser 2000 [28] | 17 RRMS 12 SPMS | – | – | 1,001 healthy subjects | 28.1 (22–32) | 49/4 | SR/ELISA |
| Höllsberg et al. 2005 [29] | 33 MS (16 TT: valaciclovir) | 33.7–38.83 | 22/11 | 7 healthy subjects | – | – | MC/PCR |
| Mancuso et al. 2007 [32] | 23 RRMS 15 chronic progressive | 34 (±10) 43 (±12) | 16/7 8/7 | 18 healthy subjects | – | – | PS/RT-PCR |
| Martin et al. 1997 [33] | 6 RRMS-AE 8 RRMS-NAE 6 SPMS | 36 (16–59) (MS patients) | 17/19 (MS patients) | 28 OND 19 NND 20 OND | 43 (±16) 70 (±10) 52 (19–83) | 16/12 2/17 13/7 | CSF/n-PCR CSF/n-PCR |
| Mýhr et al. 1998 [34] | 8 RRMS (TT: aciclovir) 3 RRMS 19 ON | 31 (20–46) (ON patients) | 15/4 (ON patients) | 170 NND | – | – | SR/ELISA and IF |
| Morré et al. 2001 [30] | 144 MS 10 MS (postmortem) | 39.2 (17–66) 53 (34–81) | 83/61 10/0 | 46 NND | 40 (18–77) 19–97 | 93/77 – | CSF and TC/PCR |

Table 1 (continued)

| Study | Cases | | | Controls | | | Sample/Test |
|-----------------------------|-------------------|----------------------------|-----------|--|----------------------------|-----------|-----------------|
| | Diagnostic | Age Mean (SD) and/or range | F/M ratio | Diagnostic | Age Mean (SD) and/or range | F/M ratio | |
| | 9 MS (postmortem) | – | – | | | | |
| | 18 MS | 38 (24–56) | 14/4 | | | | |
| Munch et al. 1998 [31] | 138 MS | 43 (22–72) | 81/57 | 138 healthy subjects | – | – | SR/ELISA |
| Pohl et al. 2006 [19] | 144 RRMS | 12.31 (±13.13) | 98/49 | 47 adrenoleukodystrophy or neuroblastoma | – | – | SR/ELISA |
| | 2 PPMS | (4.04–15.99) | | 100 ONDD | | | |
| | 1 SPMS | | | 272 healthy subjects | 43.6 (±9.2) | 184/88 | SR/ELISA |
| Ponsonby et al. 2005 [36] | 89 RRMS | 43.5 (±9.3) | 92/44 | | | | |
| | 35 SPMS | | | | | | |
| | 10 PPMS | | | | | | |
| Riverol et al. 2007 [44] | 22 ICS | 39 ± 9 | 106/66 | 85 healthy subjects | – | – | SR/ELISA |
| | 92 RRMS | | | | | | |
| | 32 SPMS | | | | | | |
| | 17 PPMS | | | | | | |
| | 9 PRMS | | | | | | |
| Sanders et al. 1996 [39] | 37 MS | 51.01 (±11.73) | 19/18 | 24 OND | 74.28 (±8.07) | 9/28 | TC/PCR |
| | | (31–70) | | 13 OND | (48–84) | | |
| Shirodaria et al. 1987 [35] | 26 MS | 51.9 (28–74) | 17/9 | 26 healthy subjects | 52 (27–74) | 17/9 | SR/IF |
| Sotelo et al. 2007 [40] | 131 RRMS-NAE | 33 (±2) | 82/49 | 60 healthy subjects | 36 (±8) | 14/46 | MC/PCR |
| | 40 RRMS-NAE | 32 (±2) | 30/10 | 70 OND and NND | 37 (±9) | 46/24 | |
| Sundström et al. 2004 [10] | 197 RRMS/SP | 17–68 | 192/2 | 702 healthy subjects | – | – | SR/IF and ELISA |
| | 24 PPMS | | | | | | |
| | 8 PRMS | | | | | | |
| | 5 NC | | | | | | |
| Sumaya et al. 1980 [37] | 157 MS | 41.42 (±12.46) | 92/65 | 81 healthy subjects | 40.68 (±13.97) | 45/36 | SR/IF |
| | | (11–>60) | | | (11–>60) | | |
| Sumaya et al. 1976 [38] | 142 MS | – | – | 74 healthy subjects | – | – | SR/IF |
| Wagner et al. 2004 [41] | 31 MS | – | 31/0 | 62 healthy subjects | – | – | SR/PCR |
| Wandinger et al. 2000 [42] | 58 RRMS-AE | 37.9 (±10.3) | 67/41 | 163 NND | 36.2 (±11.8) | 89/74 | SR/ELISA |
| | 50 RRMS-NAE | (20–57) | | | (19–56) | | |
| Zivadinov et al. 2006 [43] | 140 RRMS | 42.1 (±10.09) | 90/50 | 131 healthy subjects | – | – | SR/ELISA |

MS: multiple sclerosis; RRMS: relapsing recurrent multiple sclerosis; PPMS: primary progressive multiple sclerosis; SPMS: secondary progressive multiple sclerosis; PRMS: progressive relapsing multiple sclerosis; ICS: isolated demyelinating clinical syndromes; ON: optical neuritis; NC: not classified; NAE: no acute exacerbation; AE: acute exacerbation; TT: treatment; OND: other neurological diseases; ONDD: other non-demyelinating neurological diseases; NND: non-neurological disease; ONDD: other non-demyelinating disease; OIND: other inflammatory neurological diseases; ONIND: other non-inflammatory neurological diseases; SR: serum; PS: plasma; MC: mononuclear cells; IF: immunofluorescence; n-PCR: nested-PCR; RT-PCR: real time-PCR; F: females; M: male

Table 2 Analysis and evaluation of the quality of the studies detecting anti-VCA IgG

| Study | Descriptive statistics | | | | | | Inferential statistics | | | Quality (points) | | |
|-----------------------------|------------------------|----------|------------|----------|----------|------------|---------------------------------|-------------|------------|------------------|----|-----|
| | Cases | | | Controls | | | OR | 95% CI | Weight (%) | S | C | E |
| | Positive | Negative | Positive % | Positive | Negative | Positive % | | | | | | |
| Alotaibi et al. 2004 [17] | 25 | 5 | 83.3 | 81 | 62 | 56.7 | 3.8 | 1.38–10.56 | 11.50 | *** | * | ** |
| Ascherio et al. 2001 [22] | 143 | 1 | 99.3 | 269 | 19 | 93.4 | 10.1 | 1.33–76.22 | 4.56 | ** | ** | * |
| Banwell et al. 2007 [18] | 108 | 18 | 85.7 | 61 | 35 | 63.5 | 3.4 | 1.79–6.59 | 16.53 | * | ** | * |
| Bray et al. 1992 [23] | 56 | 41 | 57.7 | 26 | 79 | 24.8 | 4.2 | 2.28–7.55 | 17.31 | ** | | * |
| Bray et al. 1983 [24] | 309 | 4 | 98.7 | 363 | 43 | 89.4 | 9.2 | 3.24–25.77 | 11.27 | * | ** | *** |
| Myhr et al. 1998 [34] | 141 | 3 | 97.9 | 138 | 32 | 81.1 | 10.9 | 3.26–36.42 | 9.49 | ** | ** | *** |
| Pohl et al. 2006 [19] | 145 | 2 | 98.6 | 106 | 41 | 72.1 | 28.0 | 6.63–118.05 | 7.56 | ** | ** | ** |
| Ponsonby et al. 2005 [36] | 136 | 0 | 100 | 252 | 9 | 96.5 | 10.3 | 0.59–177.82 | 2.53 | ** | ** | ** |
| Shirodaria et al. 1987 [35] | 26 | 0 | 100 | 24 | 2 | 92.3 | 5.4 | 0.24–118.34 | 2.20 | * | ** | *** |
| Sundström et al. 2004 [10] | 234 | 0 | 100 | 693 | 9 | 98.7 | 6.4 | 0.37–110.80 | 2.54 | ** | ** | *** |
| Sumaya et al. 1980 [37] | 155 | 2 | 98.7 | 76 | 5 | 93.8 | 5.1 | 0.96–26.88 | 6.17 | ** | | *** |
| Sumaya et al. 1976 [38] | 140 | 2 | 98.6 | 70 | 4 | 94.6 | 4.0 | 0.71–22.37 | 5.85 | ** | | *** |
| Zivadinov et al. 2006 [43] | 133 | 7 | 95 | 131 | 0 | 100 | 0.0 | 0.004–1.19 | 2.50 | **** | ** | *** |
| Total | 1,751 | 85 | – | 2,290 | 340 | – | OR 5.5 | | | | | |
| Event rate (%) | 95.37 | 4.62 | – | 87.07 | 12.92 | – | (95% CI=3.37–8.81; $p<0.0001$) | | | | | |

OR: odds ratio; CI: confidence interval; S: selection; C: comparability; E: exposure

significant association between EBV and MS, and those by Sundström et al. [10] and Zivadinov et al. [43], which did not find any significant association. After a meta-regression to understand the relationship between the quality of the study and the corresponding OR, the said relationship was found to be significant ($p=0.017$). Since the coefficient was positive, we were able to affirm that the higher the quality of the study, higher the OR. When studying the overall OR of the articles with the highest quality, the results were similar to the initial situation, in which quality was not controlled.

Anti-EBNA complex IgG determination

In these studies [17, 22, 34, 35, 44], all of which the determination was made in serum (Table 3), the proportion of seropositive patients was higher in cases (97–100%) than in controls (88–94%). The research performed by Alotaibi et al. [17] showed the least percentage of positive cases and yet still, it was twice that of the controls; it was carried out in serum obtained from children and it was the weightiest study of the set. The study of Shirodaria et al. [35] is the only one that showed no association between the virus and the disease, while Myhr et al. [34] was the study with the highest OR supporting that association. The ORs of the studies were very homogeneous ($\chi_{exp}^2=0.70$; 3 g.l.; $p=0.874$; $I^2=0\%$). A high global OR was obtained, being 5.4 (95% CI=2.94–9.76; $p<0.0001$). The studies of

the highest quality were those by Alotaibi et al. [17] and Myhr et al. [34].

Anti-EBNA-1 IgG determination

Of these studies (Table 4), Bray et al. [23] was the only one in which the measurement was in CSF, with a very significant difference in the proportion of seropositivity in cases (79.7%) and controls (14.5%). In addition, its weight was the highest among the set. All remaining studies used serum samples [4, 10, 19, 31, 42] and the proportion of seropositive patients was also higher in cases than in controls, except for the study by Banwell et al. [18], which was carried out in children, and with an OR that failed to indicate any association between the virus and MS. The article with the highest OR was that by Haahr et al. [4].

The overall OR was the highest of all the measurements, being 12.1 (95% CI=3.13–46.89; $p<0.0001$), in spite of the great variability amongst the studies ($\chi_{exp}^2=65.76$; 6 g.l.; $p<0.0001$; $I^2=90.9\%$).

According to the data, there was significant association ($p<0.0001$) between the EBV and MS, although, due to the high variability amongst the studies, this result should be treated with caution. The studies with the highest quality were those by Haahr et al. [4] and Sundström et al. [10]. A meta-regression was carried out to study the relationship between the quality of the article and the corresponding OR, and this relation was significant ($p=0.026$) and, since

Table 3 Analysis and evaluation of the quality of the studies detecting anti-EBNA complex IgG

| Study | Descriptive statistics | | | | | | Inferential statistics | | | Quality (points) | | |
|-----------------------------|------------------------|----------|------------|----------|----------|------------|---------------------------------|-------------|------------|------------------|----|-----|
| | Cases | | | Controls | | | OR | 95% CI | Weight (%) | S | C | E |
| | Positive | Negative | Positive % | Positive | Negative | Positive % | | | | | | |
| Alotaibi et al. 2004 [17] | 25 | 5 | 83.3 | 60 | 83 | 41.9 | 6.9 | 2.50–19.10 | 49.03 | *** | * | ** |
| Ascherio et al. 2001 [22] | 141 | 3 | 97.9 | 266 | 22 | 92.4 | 3.9 | 1.14–13.21 | 33.82 | ** | ** | * |
| Myhr et al. 1998 [34] | 143 | 1 | 99.3 | 160 | 10 | 94.1 | 8.9 | 1.13–70.68 | 11.84 | ** | ** | *** |
| Shirodaria et al. 1987 [35] | 26 | 0 | 100 | 24 | 2 | 92.3 | 5.4 | 0.24–118.34 | 5.32 | * | ** | *** |
| Riverol et al. 2007 [44] | 167 | 5 | 97.1 | 75 | 10 | 88.2 | 4.5 | 1.47–13.48 | 29.21 | ** | | *** |
| Total | 502 | 14 | – | 585 | 127 | – | OR 5.4 | | | | | |
| Event rate (%) | 97.28 | 2.71 | – | 82.16 | 17.83 | – | (95% CI=2.94–9.76; $p<0.0001$) | | | | | |

OR: odds ratio; CI: confidence interval; S: selection; C: comparability; E: exposure

the coefficient was positive, we can state that the higher the quality of the article, the higher the OR. Random-effects meta-analysis of seven studies of the association between anti-EBNA1 IgG and MS is expressed in Fig. 1.

Anti-EA IgG determination

In general, these studies (Table 5) had low seropositive percentages, both in the group of cases and controls, especially in those in which both groups were constituted by child-aged subjects [17, 19]. In three of the studies, the positive proportion of positive cases was less than in controls [17, 31, 42]. The Myhr et al. [34] study showed the highest percentage of seropositivity among the cases. Only the Buljevac et al. [25] and Myhr et al. [34] studies showed a significant relationship between the virus and the disease.

The overall OR was 1.3 (95% CI=0.68–2.35; $p=0.457$) and we found no association between EBV and MS. The ORs were not homogeneous ($\chi_{\text{exp}}^2=20.30$; 5 g.l.; $p=0.001$;

$I^2=75.4\%$). The articles of the highest quality were those by Myhr et al. [34] and Wandinger et al. [42].

EBV DNA detection

Studies performed for the detection of the EBV DNA used a great variety of samples, from CSF to brain tissue biopsies, including the different components of blood. The meta-analysis was carried out using all studies that amplified the EBV DNA. In all, there was no association between EBV infection and MS ($p=0.051$). The studies were not too homogeneous, as shown by the Chi-square statistic ($\chi_{\text{exp}}^2=15.99$; 12 g.l.; $p=0.192$; $I^2=25\%$).

Detection of EBV DNA in blood

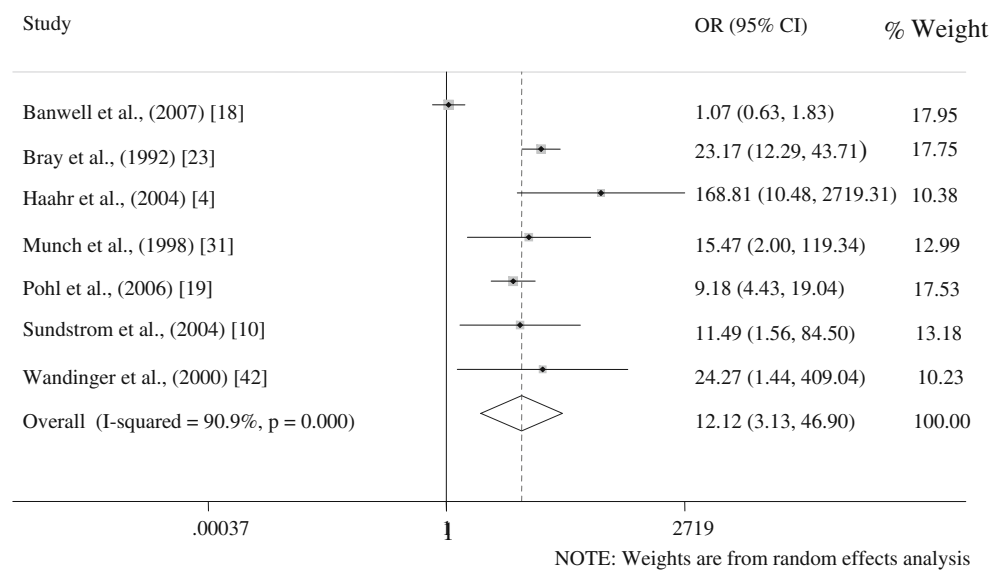
Studies based on the detection of EBV DNA in serum [41], plasma [29] and mononuclear cells [20, 27, 28, 40] (Table 6) found DNA, both in cases and in controls, but only showed

Table 4 Analysis and evaluation of the quality of the studies detecting anti-EBNA-1 IgG

| Study | Descriptive statistics | | | | | | Inferential statistics | | | Quality (points) | | |
|----------------------------|------------------------|----------|------------|----------|----------|------------|----------------------------------|---------------|------------|------------------|----|-----|
| | Cases | | | Controls | | | OR | 95% CI | Weight (%) | S | C | E |
| | Positive | Negative | Positive % | Positive | Negative | Positive % | | | | | | |
| Banwell et al. 2007 [18] | 73 | 53 | 57.9 | 54 | 42 | 56.2 | 1.1 | 0.63–1.83 | 17.95 | * | ** | * |
| Bray et al. 1992 [23] | 114 | 29 | 79.7 | 19 | 112 | 14.5 | 23.2 | 12.29–43.71 | 17.75 | ** | | * |
| Haahr et al. 2004 [4] | 153 | 0 | 100 | 646 | 355 | 64.5 | 168.8 | 10.48–2,719.3 | 10.38 | **** | ** | *** |
| Munch et al. 1998 [31] | 137 | 1 | 99.2 | 124 | 14 | 89.8 | 15.5 | 2.01–119.34 | 12.99 | | ** | *** |
| Pohl et al. 2006 [19] | 124 | 10 | 92.5 | 77 | 57 | 57.4 | 9.2 | 4.44–19.04 | 17.53 | ** | ** | ** |
| Sundström et al. 2004 [10] | 233 | 1 | 99.6 | 669 | 33 | 95.3 | 11.5 | 1.56–84.50 | 13.18 | ** | ** | *** |
| Wandinger et al. 2000 [42] | 108 | 0 | 100 | 147 | 16 | 90.2 | 24.3 | 1.44–409.04 | 10.23 | **** | | *** |
| Total | 942 | 94 | – | 1,736 | 629 | – | OR 12.1 | | | | | |
| Event rate (%) | 90.92 | 9.07 | – | 73.40 | 26.60 | – | (95% CI=3.13–46.89; $p<0.0001$) | | | | | |

OR: odds ratio; CI: confidence interval; S: selection; C: comparability; E: exposure

Fig. 1 Random-effects meta-analysis of seven studies of the association between anti-EBNA-1 IgG and MS



a significant association between the virus and the disease in the study by Sotelo et al. [40]. The study by Hay and Tenser [28] should be highlighted, with 100% positive results in both cases and controls.

The whole set of studies obtained an overall OR of 1.8 (95% CI=0.99–3.36; $p=0.051$). It could, therefore, be affirmed that no significant association was found between the virus and the disease, although the value of p was close to being significant. The ORs of the articles were homogeneous, though very close to heterogeneity ($\chi_{exp}^2=10.34$; 5 g.l.; $p=0.066$; $I^2=51.6\%$). The articles with the highest quality were those by Alvarez et al. [20] and Wagner et al. [41].

Detection of EBV DNA in brain tissue and in CSF

Studies that detected EBV DNA in brain tissue [30, 39] and CSF [21, 26, 30, 32, 33] (Table 7) failed to prove the

association between the virus and the disease. Only the results of the following studies were positive: Alvarez-Lafuente et al. [21], Mancuso et al. [32] and Sanders et al. [39]. The Sanders et al. [39] study also showed the percentage of positive results as being greater in controls than in cases. When all studies were combined, an estimated OR of 0.9 (95% CI=0.38–2.01; $p=0.768$) was obtained. The studies were rather homogeneous ($\chi_{exp}^2=3.26$; 6 g.l.; $p=0.776$; $I^2=0\%$). The article with the highest quality was that by Alvarez-Lafuente et al. [21].

Discussion

Up until now, the meta-analyses studying the possible association between EBV and MS were based on determining the OR amongst the groups made up of cases against the control groups [8], seronegative versus seropositive

Table 5 Analysis and evaluation of the quality of the studies detecting anti-EA IgG

| Study | Descriptive statistics | | | | | | Inferential statistics | | | Quality (points) | | |
|----------------------------|------------------------|----------|------------|----------|----------|------------|--------------------------------|-----------|------------|------------------|----|-----|
| | Cases | | | Controls | | | OR | 95% CI | Weight (%) | S | C | E |
| | Positive | Negative | Positive % | Positive | Negative | Positive % | | | | | | |
| Alotaibi et al. 2004 [17] | 0 | 30 | 0 | 21 | 122 | 14.7 | 0.1 | 0.01–1.59 | 4.01 | *** | * | ** |
| Buljevac et al. 2005 [25] | 26 | 28 | 48.1 | 13 | 39 | 25 | 2.8 | 1.22–6.35 | 17.47 | * | | ** |
| Myhr et al. 1998 [34] | 99 | 45 | 68.7 | 79 | 91 | 46.5 | 2.5 | 1.59–4.03 | 22.17 | ** | ** | *** |
| Munch et al. 1998 [31] | 50 | 88 | 36.2 | 57 | 81 | 41.3 | 0.8 | 0.5–1.31 | 21.92 | | ** | *** |
| Pohl et al. 2006 [19] | 9 | 138 | 6.1 | 7 | 140 | 4.7 | 1.3 | 0.47–3.6 | 15.05 | ** | ** | ** |
| Wandinger et al. 2000 [42] | 15 | 93 | 13.8 | 28 | 135 | 17.1 | 0.8 | 0.39–1.54 | 19.38 | **** | | *** |
| Total | 199 | 422 | – | 205 | 608 | – | OR 1.3 | | | | | |
| Event rate (%) | 32.04 | 67.95 | – | 25.21 | 74.78 | – | (95% CI=0.68–2.35; $p=0.457$) | | | | | |

OR: odds ratio; CI: confidence interval; S: selection; C: comparability; E: exposure

Table 6 Analysis and evaluation of the quality of the studies detecting DNA in mononuclear cells and serum

| Study | Sample | Descriptive statistics | | | | | | Inferential statistics | | | Quality (points) | | |
|----------------------------|--------|------------------------|----------|------------|----------|----------|------------|--------------------------------|-------------|------------|------------------|----|-----|
| | | Cases | | | Controls | | | OR | 95% CI | Weight (%) | S | C | E |
| | | Positive | Negative | Positive % | Positive | Negative | Positive % | | | | | | |
| | | | | | | | | | | | | | |
| Alvarez et al. 2000 [20] | MC | 33 | 69 | 32.2 | 36 | 66 | 35.3 | 0.9 | 0.49–1.57 | 28.6 | ** | ** | *** |
| Ferrante et al. 2000 [27] | MC | 13 | 13 | 50 | 7 | 11 | 38.9 | 1.6 | 0.46–5.32 | 15.11 | * | | ** |
| Hay and Tenser 2000 [28] | MC | 29 | 0 | 100 | 7 | 0 | 100 | 3.9 | 0.07–214.97 | 2.18 | | | *** |
| Höllsberg et al. 2005 [29] | PS | 5 | 28 | 15.2 | 1 | 17 | 5.5 | 3.0 | 0.33–28.23 | 6.23 | * | | ** |
| Sotelo et al. 2007 [40] | MC | 144 | 27 | 84.2 | 82 | 48 | 63.1 | 3.1 | 1.81–5.38 | 29.54 | | | *** |
| Wagner et al. 2004 [41] | PS | 9 | 22 | 29 | 10 | 52 | 16.1 | 2.1 | 0.76–5.95 | 18.34 | ** | ** | ** |
| Total | | 233 | 159 | – | 143 | 194 | – | OR 1.8 | | | | | |
| Event rate (%) | | 59.43 | 40.56 | – | 42.43 | 57.57 | – | (95% CI=0.99–3.36; $p=0.051$) | | | | | |

MC: mononuclear cells; PS: plasma; OR: odds ratio; CI: confidence interval; S: selection; C: comparability; E: exposure

patients [15] and studies linking IM with MS [14]. However, this meta-analysis tried to study the relation between all EBV infection markers to MS.

According to our study, an important increase of anti-EBNA IgG and anti-EBNA-1 IgG was found in MS patients, significantly so when compared with patients free of that disease. Other authors have highlighted the same behaviours in MS patients; it is, however, different from that found in infected immunosuppressed patients and in chronic IM, because both cases show increased anti-EBNA-2 IgG and, to a lesser degree, anti-EBNA-1 IgG [48].

This increase in EBNA complex and EBNA-1 antibodies in MS occurs between 5 and 20 years before the first symptoms of the disease appear, and continues afterwards [9, 10]. Although the cause of the high levels of antibodies is unknown, a possible explanation may be that the co-infection by other herpes viruses alters the immune control of the host on the latent EBV reactivating it, or otherwise as a consequence of a new infection by new EBV strains [49].

In addition, an autoimmune component may exist, since Cepok et al. [50] have already identified two proteins of the virus (BRRF2 and EBNA-1) that share epitope(s) with myelin basic proteins [23, 51] and against antibodies that appear in the CSF and the serum of MS patients.

Although heterogeneously, the presence of anti-VCA IgG was linked to MS, since works on the CSF determination were included [23] and using different laboratory techniques.

On the contrary, anti-EA IgG did not have a relationship to the disease, probably because the primary infection may participate in the initial stage of MS but not in the reactivation of the virus without excluding a possible autoimmune mechanism. However, in a study on 19 patients with MS exacerbation, Wandinger et al. [42] found 72.7% positivity of anti-EA IgM and IgG, the same as Buljevac et al. [25], who found an increase of anti-EA IgG in a subgroup of patients with the disease in an active phase, studied through magnetic resonance. Then, it could

Table 7 Analysis and evaluation of the quality of the studies detecting DNA in brain tissue and CSF

| Study | Sample | Descriptive statistics | | | | | | Inferential statistics | | | Quality (points) | | |
|-----------------------------------|--------|------------------------|----------|------------|----------|----------|------------|--------------------------------|-------------|------------|------------------|---|-----|
| | | Cases | | | Controls | | | OR | 95% CI | Weight (%) | S | C | E |
| | | Positive | Negative | Positive % | Positive | Negative | Positive % | | | | | | |
| | | | | | | | | | | | | | |
| Alvarez-Lafuente et al. 2008 [21] | CSF | 1 | 47 | 2.1 | 0 | 44 | 0 | 2.8 | 0.99–2.49 | 6.50 | ** | | *** |
| Denne et al. 2007 [26] | CSF | 0 | 6 | 0 | 0 | 57 | 0 | 8.9 | 0.16–484.41 | 4.22 | | | *** |
| Mancuso et al. 2007 [32] | CSF | 1 | 37 | 2.5 | 0 | 47 | 0 | 3.8 | 0.15–95.98 | 6.49 | ** | | *** |
| Martin et al. 1997 [33] | CSF | 0 | 45 | 0 | 0 | 20 | 0 | 0.4 | 0.01–23.5 | 4.33 | * | | *** |
| Morré et al. 2001 [30] | BT | 0 | 10 | 0 | 0 | 10 | 0 | 1.0 | 0.02–55.27 | 4.20 | | | *** |
| Morré et al. 2001 [30] | CSF | 0 | 27 | 0 | 0 | 36 | 0 | 1.3 | 0.03–69.01 | 4.33 | | | *** |
| Sanders et al. 1996 [39] | BT | 10 | 27 | 27 | 14 | 23 | 37.8 | 0.6 | 0.23–1.63 | 69.92 | | | *** |
| Total | | 12 | 199 | – | 14 | 237 | – | OR 0.9 | | | | | |
| Event rate (%) | | 5.69 | 94.31 | – | 5.57 | 94.42 | – | (95% CI=0.38–2.01; $p=0.768$) | | | | | |

CSF: cerebrospinal fluid; BT: brain tissue; OR: odds ratio; CI: confidence interval; S: selection; C: comparability; E: exposure

be possible that the prime infection participates at the start of MS and that the reactivation of the virus is also associated to clinic exacerbations of the disease.

In spite of all the above, the relationship between the infection and MS based on serum determinations may be masked by problems in the immune detection of the herpes viruses [49] and by false-negative results in the controls [8].

When the presence of the virus DNA in total blood, cells, plasma, CSF or brain biopsy was analysed, only one of the studies found a relationship between the infection and the disease [40]. On the other hand, it is known that EBV DNA in serum is present in most patients in the acute phase of MS, as a consequence of the virus replication; however, during the latency stage, its detection in plasma or CSF is more difficult, although the higher viral load will be found in B lymphocytes [49]. In addition, Sanders et al. [39] found a small proportion of positive cases in the brain tissue, with no differences between patients with active and inactive demyelinating plaques. Neither were there any differences when remittent or recurrent patients were considered [27, 40]. However, lytic and latent infection markers have recently been found in the CSF of MS patients, in ectopic follicles [11]. This finding suggests a new concept of the disease pathogen, where B lymphocytes would be chronically infected and become the virus carriers in the CSF.

Although the deep mechanism linking the EBV to MS is unknown, according to the above, it is possible that T and B lymphocytes generate an abnormal self-response due to cross-reaction with its own antigens that belong to the brain tissue [52]. Thus, it has been described that exposure to infectious agents induces the expression of alpha B crystalline in lymphoid cells. The immune system mistakes self alpha B crystalline for a microbial antigen and generates CD4+ T lymphocytes against it [53]. In addition, Pender [54] proposed that MS may be due to a defect in the elimination of infected B lymphocytes by CD8+ T cytotoxic lymphocytes, which may lead to their accumulation in the CNS [55].

Finally, the quality of the studies reviewed in this meta-analysis has been acceptable. Because most of our studies are not epidemiological exactly but are instead comparisons between selected individuals with MS and people without the disease, so the scale expresses lower scores in the “Comparability (C)” and “Selection (S)”. However, most articles have an average level of quality and it is acceptable. In almost all of the articles, the group of cases was selected taking into account a second medical or technical validation of the disease; however, selection was not random or consecutive, and was, thus, biased in most of the studies. For controls, in most of the articles, the selection was conducted among hospitalised patients, but MS was fully discarded in very few. On the aspect of comparison between cases and controls, almost all articles considered at least one factor: age or sex. To check for exposure to the virus, most authors employed the

high predictive value methods (serology, molecular diagnosis), using the same test for cases and controls. Specifically, on the aspect of antibody study, articles of the highest quality were those by Zivadnov et al. [43], Haahr et al. [4], Myhr et al. [34], Sundström et al. [10] and Wandinger et al. [42]. However, we may note the scarcity of comparison between cases and controls in the majority of the articles with molecular studies. In this regard, the study with the highest quality was that by Alvarez et al. [20].

In conclusion, the relationship between EBV and MS is unclear to the present date, but the virus may play an important role in the development of MS: EBV infection may be an environmental factor which, together with other environmental or endogenous factors, may alter the normal function of the immune system, in the form of autoimmunity, inflammation and demyelination. The important relationship found between the disease and anti-EBNA-1 antibodies may suggest the potential value of these antibodies as markers for early diagnostic and for follow up of MS. However, the literature analysed lacks a prospective, comparative cohort study that may determine the levels of antibodies years before the appearance of MS symptoms, so that we can obtain a definite conclusion.

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