Eur J Clin Microbiol Infect Dis (2010) 29:857–866
DOI 10.1007/s10096-010-0940-0

ARTICLE

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

O. Santiago · J. Gutierrez · A. Sorlozano · J. de Dios Luna · E. Villegas · O. Fernandez

Received: 22 November 2009 / Accepted: 3 April 2010 / Published online: 29 April 2010

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.

O. Santiago · J. Gutierrez (✉) · A. Sorlozano · E. Villegas
Department of Microbiology, School of Medicine,
University of Granada,
Avenida de Madrid, 11,
18012 Granada, Spain
e-mail: josegf@ugr.es

J. de Dios Luna
Department of Statistics and Operation Research,
University of Granada,
Granada, Spain

O. Fernandez
Neurology Service, Hospital Carlos Haya,
Málaga, Spain

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.

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 $\chi_{exp}^2 = 19.74$, 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 53 bone marrow donors	13.37 (±3.62) 10.30 (±3.78)	1.57:1 0.66:1	SR/ELISA
Alvarez et al. 2000 [20]	102 RRMS-NAE	Female: 34.75 (21–57) Male: 36.34 (22–63)	70/32	102 haemotherapy	Female: 34.75 (21–57) Male: 36.34 (22–63)	70/32	MC/PCR and n-PCR
Alvarez-Lafuente et al. 2008 [21]	48 MS	32.6	34/14	23 OIND 21 ONIND	43.7 39.4	15/8 14/7	CSF/RT-PCR
Ascherio et al. 2001 [22]	144 MS	–	144/0	288 healthy subjects	–	288/0	SR/IF
Banwell et al. 2007 [18]	131 RRMS 5 SPMS 1 Marburg variant	14.1 (2.2–19.6)	1.54:1	47 healthy subjects 49 ONDND	–	–	SR/ELISA
Bray et al. 1992 [23]	266 MS	–	–	260 OND	–	–	CSF/EIA, IF and Western blot
Bray et al. 1983 [24]	313 MS	16–>45	–	406 healthy subjects and ONDND	–	–	SR/IF
Buljevac et al. 2005 [25]	54 RRMS	39.3	–	52 healthy subjects	–	–	PS/ELISA
Denne et al. 2007 [26]	6 MS	13.3 (±2.6) (10–17)	5/1	57 OND	9.2 (±5.1)	30/27	CSF/PCR
Ferrante et al. 2000 [27]	26 RRMS-AE	31 (20–46)	17/9	18 healthy subjects	29 (19–36)	12/6	MC/n-PCR
Haahr et al. 2004 [4]	53 MS 100 newly diagnosed MS	28.3 (21–32) 27.7 (20–34)	49/4 73/27	1,001 healthy subjects	28.1 (22–32)	49/4 –	SR/ELISA
Hay and Tenser 2000 [28]	17 RRMS 12 SPMS	–	–	7 healthy subjects	–	–	MC/PCR
Höllsberg et al. 2005 [29]	33 MS (16 TT: valaciclovir)	33.7–38.83	22/11	18 healthy subjects	–	–	PS/RT-PCR
Mancuso et al. 2007 [32]	23 RRMS 15 chronic progressive	34 (±10) 43 (±12)	16/7 8/7	28 OND 19 NND	43 (±16) 70 (±10)	16/12 2/17	CSF/n-PCR
Martin et al. 1997 [33]	6 RRMS-AE 8 RRMS-NAE 6 SPMS 8 RRMS (TT: aciclovir) 3 RRMS 19 ON	36 (16–59) (MS patients) 31 (20–46) (ON patients)	17/19 (MS patients) 15/4 (ON patients)	20 OND	52 (19–83)	13/7	CSF/n-PCR
Myhr et al. 1998 [34]	144 MS	39.2 (17–66)	83/61	170 NND	40 (18–77)	93/77	SR/ELISA and IF
Morré et al. 2001 [30]	10 MS (postmortem)	53 (34–81)	10/0	46 NND	19–97	–	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 2 PPMS	12.31 (±13.13) (4.04–15.99)	98/49	47 adrenoleukodystrophy or neuroblastoma	–	–	SR/ELISA
	1 SPMS			100 ONDD			
Ponsonby et al. 2005 [36]	89 RRMS 35 SPMS	43.5 (±9.3)	92/44	272 healthy subjects	43.6 (±9.2)	184/88	SR/ELISA
	10 PPMS						
Riverol et al. 2007 [44]	22 ICS 92 RRMS	39 ± 9	106/66	85 healthy subjects	–	–	SR/ELISA
	32 SPMS						
	17 PPMS						
	9 PRMS						
Sanders et al. 1996 [39]	37 MS	51.01 (±11.73) (31–70)	19/18	24 OND 13 OND	74.28 (±8.07) (48–84)	9/28	TC/PCR
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 40 RRMS-NAE	33 (±2) 32 (±2)	82/49 30/10	60 healthy subjects 70 OND and NND	36 (±8) 37 (±9)	14/46 46/24	MC/PCR
Sundström et al. 2004 [10]	197 RRMS/SP 24 PPMS	17–68	192/2	702 healthy subjects	–	–	SR/IF and ELISA
	8 PRMS						
	5 NC						
Sumaya et al. 1980 [37]	157 MS	41.42 (±12.46) (11–>60)	92/65	81 healthy subjects	40.68 (±13.97) (11–>60)	45/36	SR/IF
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 50 RRMS-NAE	37.9 (±10.3) (20–57)	67/41	163 NND	36.2 (±11.8) (19–56)	89/74	SR/ELISA
Zivadinov et al. 2006 [43]	140 RRMS	42.1 (±10.09)	90/50	131 healthy subjects	–	–	SR/ELISA

MS: multiple sclerosis; RRMS: remitting recurrent multiple sclerosis; PPMS: primary progressive multiple sclerosis; SPMS: secondary progressive multiple sclerosis; PRMS: progressiverecurring multiple sclerosis; ICS: isolated demyelinising clinical syndromes; ON: optical neuritis; NC: not classified; NAE: no acute exacerbation; AE: acute exacerbation; TT: treatment;OND: other neurological diseases; ONDND: other non-demyelinating neurological diseases; NND: non-neurological disease; ONDD: other non-demyelinising diseases; OIND: other inflammatory neurologic 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 ($\chi_{exp}^2=20.30$; 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 ($\chi_{exp}^2=15.99$; 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

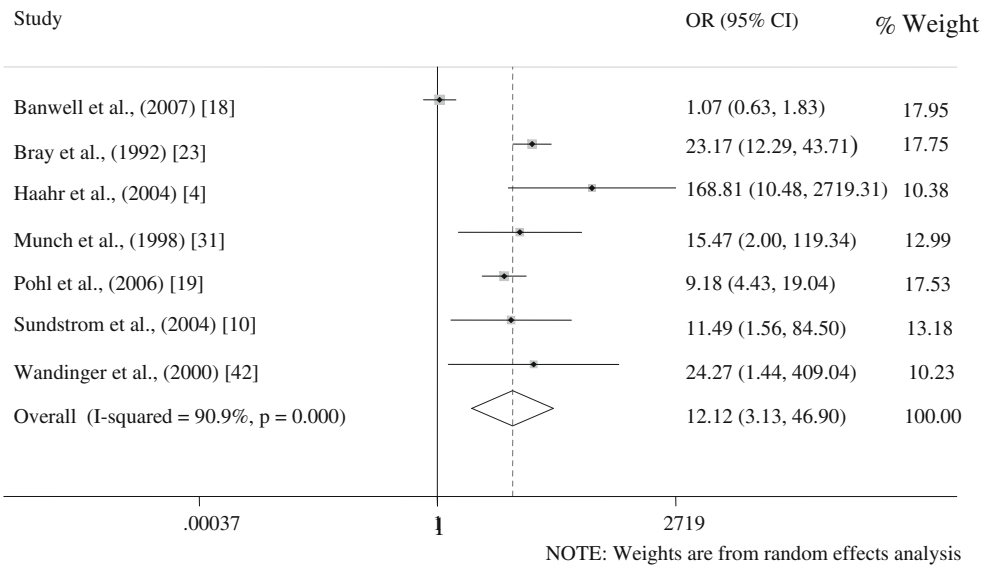

Fig. 1 Random-effects meta-analysis of seven studies of the association between anti-EBNA-1 IgG and MS

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 (χ_{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 (χ_{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 Zivadinov 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.

References

1. Fernández O, Guerrero M, Fernández VE (2005) Esclerosis múltiple. McGraw-Hill-Interamericana, Madrid
2. Haahr S, Höllsberg P (2006) Multiple sclerosis is linked to Epstein-Barr virus infection. Rev Med Virol 16:297–310
3. Salvetti M, Giovannoni G, Aloisi F (2009) Epstein-Barr virus and multiple sclerosis. Curr Opin Neurol 22:201–206
4. Haahr S, Plesner AM, Vestergaard BF et al (2004) A role of late Epstein-Barr virus infection in multiple sclerosis. Acta Neurol Scand 109:270–275
5. Haahr S, Koch-Henriksen N, Møller-Larsen A et al (1995) Increased risk of multiple sclerosis after late Epstein-Barr virus infection: a historical prospective study. Mult Scler 1:73–77
6. Hernán MA, Zhang SM, Lipworth L et al (2001) Multiple sclerosis and age at infection with common viruses. Epidemiology 12:301–306
7. Nielsen TR, Rostgaard K, Nielsen NM et al (2007) Multiple sclerosis after infectious mononucleosis. Arch Neurol 64:72–75
8. Ascherio A, Munch M (2000) Epstein-Barr virus and multiple sclerosis. Epidemiology 11:220–224
9. DeLorenze GN, Munger KL, Lennette ET et al (2006) Epstein-Barr virus and multiple sclerosis: evidence of association from a prospective study with long-term follow-up. Arch Neurol 63:839–844
10. Sundström P, Juto P, Wadell G et al (2004) An altered immune response to Epstein-Barr virus in multiple sclerosis: a prospective study. Neurology 62:2277–2282
11. Serafini B, Rosicarelli B, Franciotta D et al (2007) Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. J Exp Med 204:2899–2912
12. Thacker EL, Mirzaei F, Ascherio A (2006) Infectious mononucleosis and risk for multiple sclerosis: a meta-analysis. Ann Neurol 59:499–503

13. Ascherio A, Munger KL (2007) Environmental risk factors for multiple sclerosis. Part I: the role of infection. Ann Neurol 61:288–299
14. Holmøy T (2008) Vitamin D status modulates the immune response to Epstein Barr virus: synergistic effect of risk factors in multiple sclerosis. Med Hypotheses 70:66–69
15. Lindberg C, Andersen O, Vahlne A et al (1991) Epidemiological investigation of the association between infectious mononucleosis and multiple sclerosis. Neuroepidemiology 10:62–65
16. Marrie RA, Wolfson C (2002) Multiple sclerosis and Epstein-Barr virus. Can J Infect Dis 13:111–118
17. Alotaibi S, Kennedy J, Tellier R et al (2004) Epstein-Barr virus in pediatric multiple sclerosis. JAMA 291:1875–1879
18. Banwell B, Krupp L, Kennedy J et al (2007) Clinical features and viral serologies in children with multiple sclerosis: a multinational observational study. Lancet Neurol 6:773–781
19. Pohl D, Krone B, Rostasy K et al (2006) High seroprevalence of Epstein-Barr virus in children with multiple sclerosis. Neurology 67:2063–2065
20. Alvarez R, Cour I, Kanaan A et al (2000) Detection of viral genomes of the Herpesviridae family in multiple sclerosis patients by means of the polymerase chain reaction (PCR). Enferm Infecc Microbiol Clin 18:223–228
21. Alvarez-Lafuente R, García-Montojo M, De Las Heras V et al (2008) Herpesviruses and human endogenous retroviral sequences in the cerebrospinal fluid of multiple sclerosis patients. Mult Scler 14:595–601
22. Ascherio A, Munger KL, Lennette ET et al (2001) Epstein-Barr virus antibodies and risk of multiple sclerosis: a prospective study. JAMA 286:3083–3088
23. Bray PF, Luka J, Bray PF et al (1992) Antibodies against Epstein-Barr nuclear antigen (EBNA) in multiple sclerosis CSF, and two pentapeptide sequence identities between EBNA and myelin basic protein. Neurology 42:1798–1804
24. Bray PF, Bloomer LC, Salmon VC et al (1983) Epstein-Barr virus infection and antibody synthesis in patients with multiple sclerosis. Arch Neurol 40:406–408
25. Buljevac D, van Doornum GJ, Flach HZ et al (2005) Epstein-Barr virus and disease activity in multiple sclerosis. J Neurol Neurosurg Psychiatry 76:1377–1381
26. Denne C, Kleines M, Dieckhöfer A et al (2007) Intrathecal synthesis of anti-viral antibodies in pediatric patients. Eur J Paediatr Neurol 11:29–34
27. Ferrante P, Mancuso R, Pagani E et al (2000) Molecular evidences for a role of HSV-1 in multiple sclerosis clinical acute attack. J Neurovirol 6(Suppl 2):S109–S114
28. Hay KA, Tenser RB (2000) Leukotropic herpesviruses in multiple sclerosis. Mult Scler 6:66–68
29. Höllsberg P, Kusk M, Bech E et al (2005) Presence of Epstein-Barr virus and human herpesvirus 6B DNA in multiple sclerosis patients: associations with disease activity. Acta Neurol Scand 112:395–402
30. Morré SA, van Beek J, De Groot CJ et al (2001) Is Epstein-Barr virus present in the CNS of patients with MS. Neurology 56:692
31. Munch M, Riisom K, Christensen T et al (1998) The significance of Epstein-Barr virus seropositivity in multiple sclerosis patients? Acta Neurol Scand 97:171–174
32. Mancuso R, Delbue S, Borghi E et al (2007) Increased prevalence of varicella zoster virus DNA in cerebrospinal fluid from patients with multiple sclerosis. J Med Virol 79:192–199
33. Martin C, Enbom M, Söderström M et al (1997) Absence of seven human herpesviruses, including HHV-6, by polymerase chain reaction in CSF and blood from patients with multiple sclerosis and optic neuritis. Acta Neurol Scand 95:280–283
34. Myhr KM, Riise T, Barrett-Connor E et al (1998) Altered antibody pattern to Epstein-Barr virus but not to other herpesviruses in multiple sclerosis: a population based case–control study from western Norway. J Neurol Neurosurg Psychiatry 64:539–542
35. Shirodaria PV, Haire M, Fleming E et al (1987) Viral antibody titers. Comparison in patients with multiple sclerosis and rheumatoid arthritis. Arch Neurol 44:1237–1241
36. Ponsonby AL, van der Mei I, Dwyer T et al (2005) Exposure to infant siblings during early life and risk of multiple sclerosis. JAMA 293:463–469
37. Sumaya CV, Myers LW, Ellison GW (1980) Epstein-Barr virus antibodies in multiple sclerosis. Arch Neurol 37:94–96
38. Sumaya CV, Myers LW, Ellison GW (1976) Epstein-Barr virus antibodies in multiple sclerosis. Trans Am Neurol Assoc 101:300–302
39. Sanders VJ, Felisan S, Waddell A et al (1996) Detection of Herpesviridae in postmortem multiple sclerosis brain tissue and controls by polymerase chain reaction. J Neurovirol 2:249–258
40. Sotelo J, Ordoñez G, Pineda B (2007) Varicella-zoster virus at relapses of multiple sclerosis. J Neurol 254:493–500
41. Wagner HJ, Munger KL, Ascherio A (2004) Plasma viral load of Epstein-Barr virus and risk of multiple sclerosis. Eur J Neurol 11:833–834
42. Wandinger K, Jabs W, Siekhaus A et al (2000) Association between clinical disease activity and Epstein-Barr virus reactivation in MS. Neurology 55:178–184
43. Zivadinov R, Nasuelli D, Tommasi MA et al (2006) Positivity of cytomegalovirus antibodies predicts a better clinical and radiological outcome in multiple sclerosis patients. Neurol Res 28:262–269
44. Riverol M, Sepulcre J, Fernandez-Diez B et al (2007) Antibodies against Epstein-Barr virus and herpesvirus type 6 are associated with the early phases of multiple sclerosis. J Neuroimmunol 192:184–185
45. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7:177–188
46. Egger M, Smith GD, Altman D (1995) Systematic reviews in health care: meta-analysis in context. BMJ Books, London
47. Wells GA, Shea B, O'Connell D et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available online at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm
48. Levin LI, Munger KL, Rubertone MV et al (2005) Temporal relationship between elevation of Epstein-Barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. JAMA 293:2496–2500
49. Gutiérrez J, Sorlózano A, Soto MJ et al (2006) Microbiological diagnosis of infection by the Epstein-Barr virus: pathogenic basis. In: Umar CS (ed) New developments in Epstein-Barr virus research. Nova Science Publishers, New York, pp 277–299
50. Cepok S, Zhou D, Srivastava R et al (2005) Identification of Epstein-Barr virus proteins as putative targets of the immune response in multiple sclerosis. J Clin Invest 115:1352–1360
51. Lünemann JD, Edwards N, Muraro PA et al (2006) Increased frequency and broadened specificity of latent EBV nuclear antigen-1-specific T cells in multiple sclerosis. Brain 129:1493–1506
52. Holmøy T, Kvale EØ, Vartdal F (2004) Cerebrospinal fluid CD4+ T cells from a multiple sclerosis patient cross-recognize Epstein-Barr virus and myelin basic protein. J Neurovirol 10:278–283
53. van Noort JM, Bajramovic JJ, Plomp AC et al (2000) Mistaken self, a novel model that links microbial infections with myelin-directed autoimmunity in multiple sclerosis. J Neuroimmunol 105:46–57
54. Pender MP (2003) Infection of autoreactive B lymphocytes with EBV, causing chronic autoimmune diseases. Trends Immunol 24:584–588
55. Pender MP, Csurhes PA, Lenarczyk A et al (2009) Decreased T cell reactivity to Epstein-Barr virus infected lymphoblastoid cell lines in multiple sclerosis. J Neurol Neurosurg Psychiatry 80:498–505